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OBSERVATIONS ON A DISEASE OF FOWLS DUE TO
A FILTERABLE VIRUS AND ASSOCIATED WITH
LEUCOCYTIC INCLUSIONS.

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A DISEASE of fowls in which inclusion bodies in the leucocytes were a prominent feature was first described as occurring in Palestine by Adler¹. A similar disease, though of shorter duration, had previously been described by Macfie² in Eket, Nigeria, as long ago as 1914.

Subsequent to Adler's description of the disease, blood films from fowls were received at this Laboratory which showed definite inclusions in large numbers and were associated at times with a heavy mortality. Some of these outbreaks occurred in purely native flocks, especially in the hill districts. The incidence of the disease was erratic and for long periods it was not observed at all. An outstanding feature appeared to be a sudden wave of mortality, the remaining birds quickly recovering or to all appearance being unaffected. Owing to this peculiarity it was difficult personally to investigate individual outbreaks. On two occasions only in the last few years was it possible to do so. In these instances experiments on healthy pure-bred stock were carried out by the subcutaneous inoculation of citrated blood from a recovering bird. These experiments were either entirely abortive or did not succeed in reproducing the disease in a definite form.

The post-mortem appearances of birds which had succumbed presented fairly defined lesions. Inflammation of the intestines was mostly confined to the duodenum and was diffuse and moderate though clearly visible to the naked eye. The heart muscle was

¹Adler S. A Disease of Fowls in Palestine characterised by Leucocyte Inclusions.

²Macfie J. W. S. Quoted by Adler.

darkened and contained numerous petechiæ. The liver varied in appearance from congestion to a pale yellow and was usually friable. Frequently it showed yellowish patches or streaks which were somewhat ill-defined. The kidneys were usually congested and petechiæ were observed on the inner surface of the breast bone. The stomach was normal in appearance as a rule. In one case in which sections from the liver were examined a small-cell infiltration in and around the central veins of the lobules was noted. A constant symptom was diarrhœa, usually of a dark greenish colour but sometimes yellow. Other symptoms, though severe, were not specific for the disease. From the foregoing it was apparent that a definite disease, which for want of a better name was known as "Cell



Inclusion" or Macfie Disease, occurred amongst fowls in Palestine. One blood film from a sick turkey showing diarrhœa and fever also contained large numbers of inclusions.

Infective material was recently obtained from an outbreak in the northern part of Palestine. Of eleven hundred pure-bred poultry about two hundred succumbed. A femur and a spleen preserved in glycerine were forwarded for examination. Cultural examination from bone marrow of the femur proved negative. Some spleen material was injected intramuscularly into a pure bred leghorn (FY). This fowl died on the nineteenth day and post-mortem examination showed the lesions associated with "Cell Inclusion" disease. On examination of the blood numerous inclusions were observed in the leucocytes.

Further passages by means of spleen material were made as follows and showed a rapid increase in virulence of the original virus :—

<i>Fowl No.</i>	<i>FY.</i>	<i>FX.</i>	<i>76</i>	<i>90</i>
Died... ..	19th day	9th day	5th day	2nd day

VARIATION IN VIRULENCE OF THE VIRUS.

It soon became apparent that the increase of virulence shown in the first series of passages was not definitely maintained, and that the use of even fresh material was followed by attacks which varied from slight to severe or fatal. Material stored for three to seven days was also found to produce the same varying results. Using material which had previously been highly virulent, and which had been stored for longer periods, only mild attacks were produced. In all cases in which organ material was used for inoculation some reaction followed, the milder cases showing only slight fever. This fever, however, was frequently intermittent but continued for some time. The usual period of intermittent fever was six to eight weeks but in one case was four months. Death in fatal cases has usually taken place on the second to the fifth day.

It was subsequently found that the virulence of the virus could be raised with a fair degree of certainty if spleen or liver material was taken from a fowl freshly killed approximately at the height of the initial rise of temperature and inoculated intramuscularly into a susceptible bird.

Susceptible native fowls have been found to react similarly to pure-bred birds, but of five such fowls purchased in the market and inoculated with virulent material three resisted infection so far as visible symptoms are concerned.

The following series of passages shows the variation in virulence which occurs. The fowls used were white leghorns with the exception of FN 1, which was a native fowl.

<i>Number</i>	<i>76</i>	<i>FN1</i>	<i>93</i>	<i>96</i>	<i>100</i>	<i>3</i>	<i>6</i>
Inoculated with	Spleen	Spleen	Spleen	Spleen	Citrated blood	Spleen & Liver Filtrate	Spleen & Liver
Age of material	Fresh	Fresh	4 days	3 days	1 day	Fresh	12 days
Route	Intram.	Intram.	Intram.	Intram.	Intram.	Intram. & Subcut.	Intram.
Result	Died 5th day	Died 4th day	Died 3rd day	Lived	Killed 10th day	Died 4th day	Killed 2nd day

It was then decided to inoculate a number of fowls to estimate further the variation in virulence.

Six leghorns were purchased in the open market and were inoculated with $\frac{1}{2}$ c.c. of fresh spleen emulsion from No. 6, freshly killed. All were found dead within 24 hours. It was observed that bacteria were present in the blood of four of these birds at the time of death, the remaining two being negative, but their appearance in different birds varied and none were characteristic. A further six leghorns and three native fowls were again purchased and inoculated with spleen material from the two birds which did not show bacteria

in the blood at the time of death. These again all died within 24 hours. On this occasion bacteria were present in the blood of all the fowls but were typically bipolar. The introduction of this factor was at first puzzling, but other fowls were purchased in the same manner with the object of again investigating the matter, and at this stage the following digression must be made.

COMPLICATIONS OF CELL INCLUSION DISEASE.

Fowl Cholera.—With regard to fowl cholera it may be stated that there was at the time of writing an epidemic in and around Jaffa and the Sub-District. Previous to this outbreak very little fowl cholera had been seen in Palestine; such outbreaks as had occurred had been almost entirely sporadic and had quickly subsided. The following experiments on a further series of fowls purchased in Jaffa showed that a form of cholera was lying dormant in many of these birds, and this disease became quickly virulent when the cell inclusion organ emulsion or filtrate was inoculated. In the blood of the inoculated fowl the organisms were variable in appearance and not bi-polar or characteristic, and in some cases could not be seen in the blood at the time of death, but on subsequent inoculation from this bird to another fowl or to a pigeon they became immediately characteristic. In the filtrate experiments the filtrates were tested for sterility by broth cultures or inoculation of a pigeon or both. Syringes were sterilised by boiling for half an hour, and the same syringe was used on both the test pigeon and the fowl inoculated. A further control consisted in the inoculation of one of the fowls with filtrate from the spleen of a healthy pigeon, and in this experiment beyond a slight rise of temperature no ill effects followed. Moreover, two of the market leghorn fowls which were inoculated subcutaneously with living staphylococci in the course of other work, and one fowl which received a strain of fowl typhoid of doubtful virulence, remained unaffected. In no case did any of the locally purchased fowls die or show clinical symptoms apart from those inoculated with the cell inclusion filtrate. Pure bred fowls also, which were obtained from the Stud Farm, Acre and bred under conditions of isolation, did not succumb to cholera on being inoculated with cell inclusion filtrate.

The following table shows the results of inoculation of fowls purchased locally in the open market:—

	<i>Died of Cholera.</i>	<i>Un- affected.</i>	<i>Total.</i>
Inoculated with blood or organ emulsion from fowl affected with cell inclusion disease	6	3	9
Inoculated with filtrate from organ emulsions	2	2	4
Not inoculated	0	5	5

In these cases it would appear that the cell inclusion virus had the immediate effect of raising the virulence of a strain of fowl cholera which was lying dormant and causing no symptoms. With the exception of a few previously infected birds, all remaining fowls were disposed of and the cages were cleaned and disinfected. Fowls for further experiments were subsequently only obtained from clean and isolated premises, and no further cases of cholera occurred.

Fowl Typhoid.—In two instances in which a heavy mortality had occurred amongst fowls the blood examination showed typical cell inclusions, but *B. sanguinarium* was also recovered in pure culture from the bone marrow. One of these outbreaks was confined to native poultry in a village, and in a few weeks some 400 birds died out of a total of 600. In the other case 15 per cent. of the fowls died after vaccination with fowl typhoid vaccine, an untoward result not previously experienced in the use of our vaccine in fowl typhoid outbreaks.

Spirochaetosis.—Cell inclusion disease has also been observed in conjunction with spirochaetosis.

FILTRATION EXPERIMENTS.

I.—Fowl No. 81.

On November 3rd, 1930, this bird was inoculated with 2 c.c. liver filtrate from FX., which had died the previous day, the liver having been stored in glycerine and saline.

Filtration was carried out at room temperature, using a Seitz (Uhlenhuth) filter with a No. 6 EX disc, under direct pressure. The actual filtration occupied not more than five minutes and the filter was subsequently tested with a virulent culture of *B. avisepticus* and pigeon inoculation.

Fowl 81 passed through a severe attack. On the day following inoculation the temperature rose to 110.6° F. and did not fall below 108° F. until the ninth day. For a period of 47 days an irregular fever was shown, the temperature falling to normal on two days only; on the thirty-fifth, thirty-ninth, and forty-first days it was 109° F. or more.

The blood was examined on January 6th, 1931, four days after inoculation, and inclusions were found in the polymorphonuclear leucocytes. On November 8th, 1930 there appeared to be a leucocytosis. A count of 100 leucocytes showed 63 per cent. to be polymorphs, of which 38 per cent. contained inclusions. On the following day the number of leucocytes seemed to have diminished and only a few polymorphs contained inclusions. The next day the blood appeared normal.

Examination was continued until November 25th, 1930, but no more inclusions were observed.

II.—Fowl No. II.

Was inoculated intramuscularly on December 29th, 1930, with 2 c.c. spleen material obtained from Fowl 7 and passed through a Berkefeld filter. Fowl 7 was killed when showing a temperature of 109.2° F. and when inclusions were present in the blood.

Blood films from Fowl No. 11 were examined on January 1st, 1931, four days after inoculation, and typical inclusions were found in most of the polymorphs. On January 8th, 1931, only a few cells were found to contain inclusions and by January 15th, 1931, they had practically disappeared. During the above mentioned period no increase of leucocytes was apparent.

The temperature remained normal until January 1st, 1931, the sixth day after inoculation, when it rose to 109.6° F. Marked diarrhoea of a light yellow colour was observed and the bird appeared very depressed, remaining in a sitting position with ruffled feathers.

On the following day the temperature dropped to normal and it remained below 108° F. until February 10th, 1931, the forty-fourth day, when it rose to 108.2° F. The symptoms which had abated in the interval reappeared

and examination of the blood showed a relative increase in the polymorphs, 65 per cent. of which contained inclusions.

Unfortunately, no time was available for further examination of the blood of Fowl 11 until February 20th, 1931. From this date daily examinations were carried out until March 24th, 1931, the eighty-eighth day after inoculation. During this period the temperature never rose above 107.2° F. and on the fifty-fifth, fifty-eighth, sixtieth, sixty-second, sixty-third, sixty-fourth, sixty-sixth, seventieth, and seventy-second days several polymorphs were found to contain inclusions.

III.—Fowl No. 60.

Was inoculated intramuscularly on February 9th, 1931, with 4 c.c. spleen and liver filtrate from Fowl 59. The latter bird had been killed on the day following inoculation, when it showed fever symptoms and inclusions in the blood.

The filtrate was obtained by means of a Seitz (Uhlenhuth) filter, No. 6 EK. special disc, using negative pressure of 15 inches mercury with a Geryk pump. The time of filtration occupied five minutes and was carried out at room temperature.

A pigeon inoculated with $\frac{1}{2}$ c.c. of the filtrate remained unaffected and inoculated broth tubes remained sterile after four days incubation.

On February 10th, 1931, the day following inoculation, the temperature was 108.8° F. and diarrhoea and marked depression were noted.

Examination of the blood revealed an apparent increase of polymorphs and a count of 100 showed 88 per cent. to contain typical inclusions. The temperature on the third day was 109° F. but thereafter subsided and the bird remained normal in appearance.

As in the case of Fowl 11, circumstances prevented further examination until February 20th, 1931. From this date blood examination was carried out daily until March 25th, 1931, the forty-fifth day after inoculation. During this period the highest temperature recorded was 106.8° F., and on the twenty-second and thirty-seventh days one polymorph with typical inclusions was observed. On several other days the polymorphs contained the dot-like bodies subsequently referred to (page 164).

IV.—Fowl No. 62.

On February 13th, 1931, this bird was inoculated intramuscularly with 4 c.c. filtrate of mixed spleen and liver emulsion from Fowl 61, killed during the initial febrile period. The material was diluted 1 to 4 in saline, making a total of 25 c.c., and prior to filtration 5 c.c. of a tested virulent broth culture of *B. avisepticus* was added.

Filtration was affected with a Pasteur Chamberland L.5 candle under a negative pressure of 15 inches mercury at room temperature, and 5 c.c. filtrate was obtained in 20 minutes. A pigeon inoculated with 0.5 c.c. filtrate remained unaffected and inoculated broth tubes were sterile after seven days incubation.

On the day following inoculation the temperature rose to 108.4° F. and diarrhoea and general depression were observed. On February 15th, 1931, the third day after inoculation, inclusions were found in practically every polymorph. The next day a count of 100 polymorphs showed 75 per cent. to contain inclusions, but on the fifth day the polymorphs had apparently increased in numbers and inclusions seemed to have decreased, in 50 fields only eight polymorphs containing inclusions. The following

day and up to the fourteenth day the temperature did not rise above 106.2°F . and no inclusions were seen, but on the eighteenth day the temperature in the morning was 107.4°F . and in the evening rose to 108.2°F ., and in 50 fields six polymorphs were found to contain inclusions. From the eighteenth to twenty-fourth day the temperature remained above 107.6°F . and inclusions were constantly present. From the twenty-fourth day blood examination was carried out until the thirty-eighth day, but no further inclusions were observed. During this period the temperature did not rise above 106.8°F . except on the thirty-seventh day, when 108.8°F . was recorded.

V.—Fowl No. 67.

Was inoculated intramuscularly on February 26th, 1931, with 4 c.c. filtrate of spleen and liver obtained from Fowl 69.

The material was ground up with sterile sand and emulsified in normal saline, and 5 c.c. broth culture of *B. avisepticus* was added, making the final dilution 1:10. A pigeon inoculated subcutaneously with 0.25 c.c. of this emulsion died the next morning from fowl cholera.

Filtration of the remaining emulsion was carried out at room temperature, using a Pasteur Chamberland L.3 candle at a negative pressure of 15 inches mercury. In 12 minutes 6 c.c. filtrate was obtained. From this filtrate tubes of broth were sown and a pigeon received 0.5 c.c. subcutaneously and a fowl (67) 4 c.c. intramuscularly. The tubes of broth remained sterile and the pigeon unaffected.

On February 27th, 1931, the day following inoculation, the temperature of Fowl 67 rose to 109°F . and at noon was 110.2°F . Diarrhœa streaked with blood was observed and the bird remained standing perfectly still with head drawn in, tail drooped, and feathers ruffled. Blood films were examined and in 100 fields twelve polymorphs were seen, eleven of which contained inclusions. On the third day the temperature was 108°F ., the symptoms still persisted, and in 100 fields 32 polymorphs all of which contained inclusions were observed. The temperature recorded on the fourth day was 108.6°F . A leucocytosis was apparent and polychromasia noticeable. Practically all the polymorphs contained inclusions. The next day the bird was found sitting in the cage and was unable to stand when placed on its feet. It died at 9 a.m. that morning.

THE LEUCOCYTIC INCLUSIONS.

On examining the blood films taken from infected fowls chromatin staining inclusions were observed in the leucocytes (see Fig., page 158). These inclusions varied in shape and size and could be classified into the following types:—

1. Solid spherical masses.
2. Regular rings, and in some cases incomplete rings.
3. Irregularly shaped masses.
4. Minute granules which were sometimes situated in vacuoles.

With the exception of two or three instances when the large mononuclears were affected, the inclusions were found only in the polymorphonuclear leucocytes. The cytoplasm was most commonly affected but occasionally the nucleus also contained inclusions. The numbers varied from a few to very many. Sometimes the

whole of the cytoplasm was occupied, and occasionally a leucocyte was seen which contained only inclusions, the nucleus having disappeared. Less frequently a disintegrated cell was observed with inclusions lying free in the plasma.

The solid and ring types were most commonly found, the granules in vacuoles being more rare, but all types have been observed in the one cell.

Blood Changes.

Polychromasia, although not a constant feature and as a rule not pronounced, was observed in quite a large percentage of cases.

Erythrophagocytosis was observed in only one case and in that instance was of a very severe nature.

Staining.

The nuclei of infected polymorphs did not stain so deeply as usual and the cytoplasm often appeared vacuolated.

A peculiar staining reaction of a number of unaffected polymorphs was observed. The cytoplasm of these cells either stained light blue or pale red and had a homogenous appearance. The nucleus had a very clear-cut aspect, being separated into two, three, or four round or pear-shaped parts and staining deep blue. These cells were observed in practically every case, but no conclusion was arrived at as to their connection or otherwise with the disease.

In several cases, particularly of the mild type of the disease associated with intermittent fever and periodic leucocytosis, dot-like bodies were found in the cytoplasm. These were similar to the solid type of inclusion but were smaller and more regular in size. They did not, however, show the characteristic deep staining associated with a typical inclusion and were not considered definite enough to be identified as such.

DESCRIPTION OF THE DISEASE IN EXPERIMENTAL CASES.

(a) Period of Incubation and Temperature.

In severe and fatal cases the incubation period was usually very short, a rise of temperature occurring in 24 hours. In fatal cases the rise was abrupt, the temperature on the day following inoculation being in the vicinity of 109° F., finally rising to 110° F. or more, and death usually occurred in 2 to 5 days. The accompanying charts show the early rise of temperature in fatal cases.

In severe, as compared with fatal, cases the fever curve was more gradual.

In mild cases except for a low intermittent fever no symptoms were observed.

The usual period of fever in recovering cases is long, averaging six to eight weeks. During this period intermittent rises of

temperature are noticed and exacerbations or relapses occur, with recurrence of symptoms.

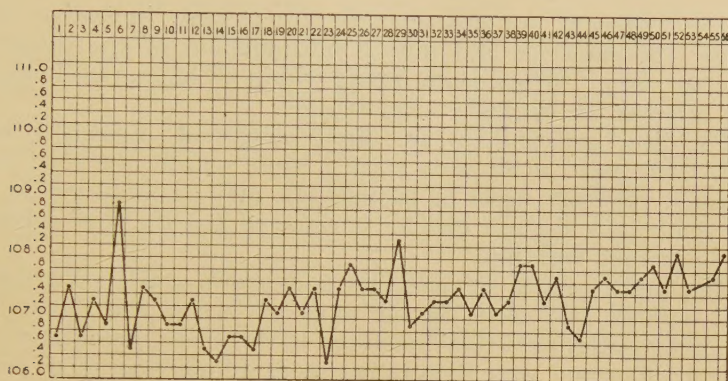


Chart of Fowl 89.

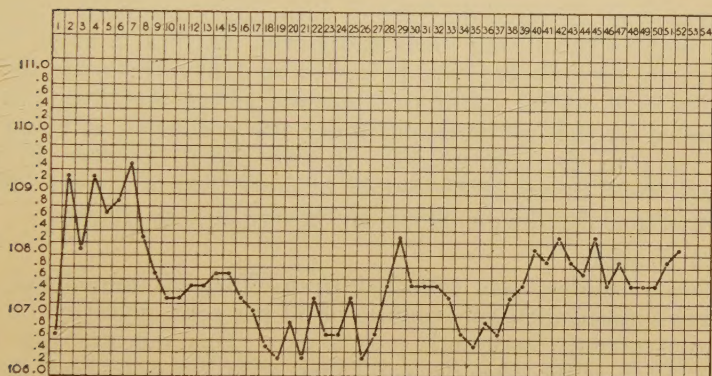


Chart of Fowl 96.

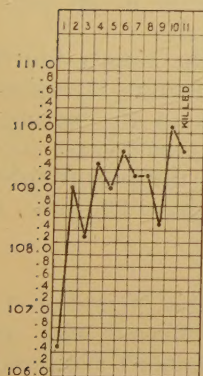


Chart of Fowl 100.

(b) Symptoms.

In severe cases the bird does not feed, becomes depressed, and the feathers stand out. Diarrhœa is a prominent symptom and is variable in colour. In many cases it is whitish or yellow but may be of a dark green colour, especially in the more severe types of the disease. Respiratory symptoms are not noticed, nor in most cases is there marked increase of exudate in the mouth. There is no discharge from the nostrils. Before death cyanosis of the comb and wattles occurs. Slight cases appear to be normal and would pass unnoticed were temperatures not taken.

(c) *Mortality and Lesions.*

In the experimental cases the mortality has been about 25 per cent.

The lesions if not characteristic are at least indicative of the disease. A moderate and diffuse inflammation of the duodenum is usually seen. When death occurs within 36 hours the duodenum may, however, be markedly hæmorrhagic.

The liver and kidneys are often congested, especially where death takes place at an early stage. More usually the liver shows elongated yellowish areas or patches but may be quite yellow. In very acute cases the gall bladder is enlarged and the liver substance may be stained with bile.

The heart muscle is darkened and shows small petechiæ. A small number also occur on the inner surface of the breast bone. In only two cases have they been seen in the lining membrane of the proventriculus and were then rather more diffuse than those seen in fowl plague.

ROUTES OF INFECTION AND DISTRIBUTION OF THE VIRUS.

In view of the rapid decrease in virulence of the virus which took place both in recovering fowls and stored material, a large number of the pure-bred fowls available had to be used to maintain the virus or raise its virulence. For this purpose spleen and liver emulsion inoculated by the intramuscular route was preferred, as being more likely to give the best results. In two cases, however, in which citrated blood drawn at the height of fever from a severely affected bird was inoculated subcutaneously, severe attacks ensued.

Mouth Exudate.

Mouth exudate was collected from Fowl 76 immediately after death and mixed with ten volumes of 50 per cent. glycerine in saline, the total quantity amounting to 3 c.c. This mixture was stored in the ice chest for three days and then given *per os* to Fowl 89. On the sixth day the temperature rose to 108.8° F. and a greenish diarrhœa was observed and continued for two days. An intermittent fever without other symptoms ensued over a period of 66 days, when the recording of temperatures was discontinued.

Blood examination was carried out daily until the twenty-fourth day and thereafter at intervals; at no time were definite inclusions observed. On the fifth day an apparent increase in the number of leucocytes was noted and it lasted until the twenty-first day. On several occasions dot-like bodies were seen in the polymorphs, which, however, were not definite enough to be identified as inclusions.

Contact.

Fowl No. 88 was placed in a small cage first with No. 90 and on the death of this bird with Fowl FN 2, both being affected with the disease. On the eighth day after being placed in contact symptoms of depression and diarrhœa occurred, the temperature rising to

109° F. and remaining above 108° F. until the twelfth day. During this period there was an apparent leucocytosis and inclusions were observed in a few polymorphs on the ninth, tenth, eleventh, and twelfth days. The visible symptoms abated but an intermittent fever continued. Daily examinations of the blood were carried out until the thirty-ninth day but no inclusions were seen. On February 22nd, 1931, approximately 3½ months after the commencement of the experiment, the temperature rose to 108·8° F. and inclusions were found in four of the polymorphs. A slight diarrhœa occurred and the bird died a few days later. Post-mortem examination showed patchy yellowish areas in the liver, which was soft and friable. The spleen was slightly enlarged and there were numerous whitish areas on the surface. The kidneys were swollen and contained pus. The duodenum and rectum showed a moderate hæmorrhagic inflammation.

LONGEVITY AND VIRULENCE OF THE VIRUS.

Spleen and liver were freshly removed from Fowl 76 and stored in 50 per cent. glycerine and saline in the ice chest at an approximate temperature of 52° F. This bird had been inoculated on November 2nd, 1930, and died on the fifth day from an acute type of the disease. Intramuscular inoculations into healthy pure-bred fowls, as shown in the following table, were made at intervals over a period of 2½ months. The tests show that a progressive diminution in virulence took place. Fowl 12 was scarcely affected but was found subsequently to be immune. No. 53 was not clinically affected, but an immunity test could not be carried out when attempted later as it was found that the virus had died out in the material in our possession.

SURVIVAL OF VIRUS.
MATERIAL FROM FOWL 76 (died on November 6th, 1930).

No. of Fowl.	Date of Inoculation.	Inoculum.	Age of Material.	Result.	Blood Examination.	Remarks.
FN 1...	6.11.30	Spleen and Liver	Fresh	Died 4th day	Inclusions found	
FN 90	9.11.30	Spleen and Liver	3 days	Died 2nd day	Inclusions found	
FN 92	11.11.30	Spleen and Liver filtrate	5 days	Mild attack	Inclusions found	
FN 101	14.11.30	Spleen and Liver	8 days	Mild attack	Negative	
FN 4	8.12.30	Spleen and Liver	32 days	Mild attack	Negative	
FN 12	29.12.30	Liver	53 days	Doubtful attack	Not examined	Tested with virulent material on 15.2.31, was found to be immune.
FN 53	20.1.31	Liver	75 days	Clinically unaffected	Not examined	Not tested for immunity

IMMUNITY TESTS.

Fowl 91 was inoculated on October 10th, 1930, and passed through a severe attack which was followed by an intermittent fever. This continued until January 19th, 1931. It remained normal until February 16th, 1931, when it was re-inoculated with material from Fowl 63, killed at the height of fever. No fever or other symptoms were observed as a result of this re-inoculation. The same material taken from Fowl 63 was stored for eight days and used on a healthy bird to maintain the virus. It was found to be virulent.

Fowl 98 was inoculated on November 27th, 1930, and passed through a mild attack, exhibiting a low intermittent fever until January 10th, 1931, from which date the temperature remained normal. It was re-inoculated on February 16th, 1931, with the same material taken from Fowl 63. It resisted infection.

Fowl 12 had been inoculated on December 29th, 1930, with a Berkefeld filtrate from Fowl 76, the filtrate having been obtained from material which was already 53 days old. A doubtful attack followed, the temperature only rising slightly on two occasions, and the blood was not examined. On February 15th, 1931, it received intramuscularly 2 c.c. of citrated blood, also from Fowl 63. It remained unaffected.

DIFFERENTIAL DIAGNOSIS.

The leucocytic inclusions which are present in large numbers in the blood in all well-marked cases clearly separates this disease from other common virus diseases of fowls. The post-mortem lesions whilst perhaps not specific are fairly regular in appearance and are a considerable aid in diagnosis. The rapidity with which the disease disappears under natural conditions and its tendency to flare up rather than spread in the manner usual in other virus diseases of fowls are also points of differentiation.

With regard to infectious leukæmia, the variation in virulence of the virus appears to admit of a possible relationship, but no marked inclusions in the leucocytes seem to have been described. Moreover, in this disease the predominance of large mononuclears is mentioned (Kaupp), whereas in cell-inclusion disease any increase occurs in the polymorphs, and in more than 99 per cent. of cases the inclusions are found only in polymorphs. Also, even in the slightest attacks, the incubation period has been short, whilst that of infectious leukæmia has an incubation period of one to two months (Hutyra and Marek).

REMARKS.

The disease, first observed by Macfie and later by Adler, was ascribed by them to "true parasites belonging probably to the Chlamydozoa" the inclusions being causally related to the disease. It is difficult to reconcile this hypothesis with the general course of the disease as shown in our experiments. It is to be noted that Adler succeeded in transmitting the disease with a Berkefeld filtrate

but deduced from this experiment that the more minute granules were the actual infective agent since "the other forms of inclusions are too large to pass through a Berkefeld filter."

Until, however, the rôle of inclusion bodies in virus diseases is more clearly understood it would seem more logical to group the causal agent of this disease with other ultra-visible viruses until the contrary can be proved.

CONCLUSIONS.

1. The disease of fowls previously described by Macfie and Adler, which is not uncommon in Palestine, is caused by a filterable virus.
2. The disease is nearly always associated with leucocytic inclusions.
3. A great variation occurs in the virulence of the virus.
4. The virulence can be raised by rapid passage.
5. A period of immunity follows both acute and mild attacks.
6. Native flocks are at times susceptible to acute outbreaks.

THE INFECTIVITY OF THE BLOOD IN ARTIFICIAL AND NATURAL CASES OF BOVINE PLEURO-PNEUMONIA.

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On perusal of the literature on Contagious Bovine Pleuro-pneumonia, it is a striking fact that the medium always referred to for the isolation of the organism is lymph—either in the thoracic cavity of a natural case or in the œdematous subcutaneous swelling in artificial cases. There appears to be a limited amount of information obtainable as regards the infectivity of the blood; it is generally accepted that the organisms are seldom present in the blood stream, that propagation does not take place there, and that the virus may be carried to other parts of the body, but there is no mention of the various organs from which the virus can easily be cultivated.

Hutyra and Marek in their classical work on Pathology state that the “circulating blood does not appear to be favourable for the propagation of the virus, as blood from infected animals is usually not infectious” and “exceptionally however the blood may contain the virus and disseminate it through the body.”

Kelser, in his “Manual of Veterinary Bacteriology” states of the virus “only rarely can it be demonstrated in the circulating blood.”

Others assume that the virus circulates in the blood after vaccination and suggest the possibility of vaccination setting up lung infections.

For the collection of lymph from the subcutaneous œdematous swelling of an animal reacting to an inoculation of culture or virus the life of the reactor has usually to be sacrificed, though in many cases recovery would take place spontaneously. The possibility of maintaining strains of virus in animals by the sub-inoculation of blood drawn during the height of the local reaction suggested itself to us as a more satisfactory and easier method and the following experimental inoculations were made.

I.—TRANSMISSION OF INFECTION BY BLOOD INOCULATION.

Bovine 1955 gave a typical reaction locally to an inoculation of culture subcutaneously. When the swellings had assumed marked proportions the animal was bled 25 c.c. into an equal quantity of 5% sodium citrate solution.

Bovine 2020. Received the whole of this citrated blood subcutaneously behind the shoulder. On the fifth day a hard nodule was present which increased daily until by the tenth day it had extended down to the forearm. The animal died from the reaction on the nineteenth day.

On the eleventh day, when the temperature was 104.4°, this

animal was bled from the jugular into citrate solution and Bovine 2208 was inoculated.

Bovine 2208 was inoculated subcutaneously behind the shoulder with 20 c.c. of this citrated blood. On the sixth day there was a painful subcutaneous nodule which increased enormously for fourteen days and then began to subside, and after a prolonged period the animal recovered.

On the seventeenth day, when the swelling was still extensive but the temperature dropping from a maximum of 105°, blood was withdrawn into citrate solution for injection into Bovine 2285.

Bovine 2285 was inoculated subcutaneously with 2 c.c. The temperature was above normal on the fifth day only, and there was no local swelling for three weeks, when the animal was destroyed. Blood taken on the fifth day and inoculated into Bovine 2251 failed to produce a reaction.

Conclusion.—The two sub-inoculations which proved positive were sufficient to show that the virus of pleuro-pneumonia may be transmitted by blood-inoculation.

The non-reactor may possibly have been immune or the blood drawn at the late stage of the infection non-infective.

II.—THE ISOLATION OF THE VIRUS IN PURE CULTURE FROM THE BLOOD.

Bovine 2020 whose reaction is described above was bled directly into tubes of Martin's bouillon on the same day as the blood was withdrawn for inoculation of Bovine 2208 and all tubes gave a positive growth.

Bovine 2208.—On the eleventh day after inoculation blood was withdrawn from the jugular directly into the above media and positive cultures of the virus of pleuro-pneumonia resulted.

Conclusion.—Pure cultures of the virus were obtained from circulating blood in Martin's bouillon.

III.—AT WHAT PERIOD DOES THE BLOOD BECOME INFECTED IN CASES OF ARTIFICIAL INFECTION WITH THE VIRUS OF PLEURO-PNEUMONIA AND FOR HOW LONG DOES IT RETAIN THE INFECTION ?

In this series of experiments all cultural work was performed on animals artificially infected, and the investigations were mainly confined to demonstrating the period of infectivity of the blood and the infection of the various organs of the body.

Cultures obtained from the blood grew well on the media generally used for the cultivation of this organism and no differences were observed in various generations in the virulence of the organisms when compared with cultures obtained from lymph.

The following were the observations recorded :—

Bovine 2161.—On January 28th, 1930, was inoculated subcutaneously behind the shoulder with a pleuro-pneumonia culture three days old and of the tenth generation. Blood was drawn the following day from the jugular with strict aseptic precautions direct into tubes

containing Martin's bouillon plus ox serum, and each succeeding 24 hours fresh tubes were inoculated in a similar manner and then incubated.

On the fourth day after inoculation a slight swelling was noticed at the site of inoculation, and the following day it had increased and measured 3-in. by 2-in. By the seventh day the swelling was 6-in. by 4-in. and was extending towards the axilla.

The temperature on the seventh day was 102.2° , which was the first time that a temperature above normal was registered. The swelling increased in size each day and the temperature gradually rose to 105° on the eighteenth day, which was the maximum recorded. The animal succumbed on the twenty-third day as a result of intoxication due to the infection. The swelling by this time had extended down the whole limb and involved the dewlap.

Blood cultures which had been made on each day up to the eighth proved to be negative. On the tenth day of culturing, however, a contamination resulted, and all subsequent cultures for the following seven days were contaminated. A further batch of medium was inoculated and a contamination again occurred, and, though further attempts were made and the technique slightly altered, it was found impossible to obtain a pure culture from the blood of this animal. The cultures, however, did show that up to the eighth day after inoculation, the blood did not contain the virus. Control tubes inoculated with the virus of pleuro-pneumonia gave a good growth.

Bovine 2039 was inoculated with 4 c.c. of a pleuro-pneumonia culture, fourth generation and four days old, and on the sixth day a swelling was observed, which gradually increased in size until the animal succumbed to the infection on the eighth day.

The first thermal reaction was recorded on the ninth day, when the temperature was 102° , and the maximum temperature of 104° was on the fifteenth day.

Tubes were inoculated from the jugular on the sixth, seventh, and eighth days and all proved to be sterile.

On the ninth day a positive culture was first obtained, and on each of the following six days the results were all positive.

The first growth in Martin's bouillon was not noticed in the usual period of three to four days, but on the seventh day of growth peculiar comet forms were observed.

These comet-like forms were bodies floating in the medium and were too numerous to count. The medium itself was jelly-like. In the lower portion of the medium the blood had settled and the medium for about $\frac{1}{4}$ -in. from the bottom of the tube was clear and liquid. There was a marked line of demarcation between the clear portion of the medium and the portion which was gelatinised. Each comet-like body was isolated, and, as it was greyish-white in colour, the jelly-like portion of the medium resembled a heavy growth seen in a gelatine shake culture.

The other tubes inoculated with the blood of the same animal on the following six days did not show these bodies; the growth in these produced only the usual faint cloudiness so characteristic of a positive growth of the pleuro-pneumonia organism when grown in Martin's bouillon with the addition of serum.

Two oxen were inoculated with 2 c.c. of the culture showing the comet-like bodies, and one reacted with a slight swelling on the sixth day after inoculation; the swelling increased until the animal was destroyed, and a post-mortem showed the characteristic lesions of artificial pleuro-pneumonia. The other ox proved to be immune.

A sub-inoculation was made from the original culture showing the comet bodies into fresh tubes of Martin's bouillon and a positive growth was obtained in 72 hours, but the comet bodies were not observed again in this or any other of the sub-cultures. Smears of these bodies stained with various stains did not reveal anything characteristic when examined under a magnification of 1,200.

Bovine 2263 was inoculated in the left side with virulent culture and kept under observation for four weeks, during which time its temperature was taken daily. A local reaction was never observed during this period, and the temperature on only two occasions, the seventeenth and the twenty-second day, registered above normal, and then came down to normal the following day on each occasion.

Blood culture on the seventeenth day proved negative; the animal was undoubtedly immune.

Bovine 2106 also proved an unsuitable subject owing to being immune. It was inoculated on the left side with 1 c.c. culture, and it ran an abnormal temperature from the day following the inoculation. For twelve days its temperature ran between 103° to 103.5°. Blood and gland smear examinations were negative. Local reaction was never observed and blood cultures were also negative.

Bovine 2449 was inoculated with 1 c.c. P.P. culture subcutaneously on the left side. On the fourth day after inoculation a slight painful swelling was observed, and it had increased to 3-in. by 2-in. on the seventh day after inoculation.

On this day the first rise of temperature was noted, 102° F. Blood was drawn from the jugular and inoculated into tubes of ox broth and peptone with the addition of horse serum, with the result that a positive growth of P.P. was obtained. On the following day the swelling at the site of inoculation was slightly œdematous and extending downwards. Blood from the jugular was again drawn and inoculated into culture medium with a positive result. On the twelfth day after inoculation a positive culture was again observed from the blood. The temperature was now 101°, and it rose to 103° on the nineteenth day, when a culture again proved positive. From the twenty-second day the swelling resulting from the inoculation began to subside and the animal eventually made a recovery. The temperature dropped to 102° on the twenty-second day and fell

to 100.4° on the twenty-seventh day ; cultures were negative on the latter day and also before the animal was discharged.

Bovine 2633 was inoculated on August 21st, 1930, subcutaneously with 1 c.c. of P.P. culture. The first indication of swelling was on the fifth day after inoculation and on the eighth day it was 3½-in. by 2-in. and accompanied by the first rise of temperature, 102°. A jugular culture proved negative, and so were the other cultures previously taken on the third and sixth day. On the ninth day the temperature dropped to normal and again a jugular culture was negative. On the tenth day the temperature rose to 103°, and on the eleventh the swelling, which had increased to 7-in. by 3-in., was flat, hard, and extending downwards towards the elbow. On the twelfth day the swelling had increased to 10-in. by 6-in. and a blood culture was positive. The swelling continued to increase while the temperature remained above normal, and a culture made on the fourteenth day was again positive. On the twenty-second day the temperature dropped to 99.2°, and the swelling was more extensive, involving the dewlap. A blood culture was again positive. The animal was destroyed *in extremis* three days later. Pure cultures from the spleen and prescapular glands were obtained.

Bovine 2707 was inoculated with 1 c.c. of P.P. culture subcutaneously on August 21st, 1930. On the eighth day after inoculation a small flat swelling was noticed ; the temperature was still normal and continued to remain so for fourteen days after the date of inoculation. A blood culture made on the twelfth day, when the local reaction was 9-in. by 12-in. but the temperature still normal, proved positive on the thirteenth day of incubation. On the fourteenth day, the first day of rise of temperature to 102.2°, a blood culture was positive after seven days of incubation. An increase in the number of organisms in the blood no doubt accounted for the quicker growth in culture medium. On the twenty-third day the temperature rose to 104°, and the swelling by this time had involved the chest wall and was extending towards the limbs and dewlap. From a jugular culture on this day a growth was obtained after 72 hours incubation. On the thirtieth day the animal was unable to rise, its temperature was 105° and the swelling extensive, and a blood culture then made was positive after 72 hours incubation. The animal died four days later. Blood cultures up to the day before death were positive, and positive cultures were also obtained from the spleen, liver, kidney, heart, and prescapular, precrucial submaxillary, mediastinal, and mesenteric glands.

Bovine 2262.—Inoculated on the left side subcutaneously with 1 c.c. P.P. culture on August 21st, 1930. At no time in a period of four weeks was a local reaction observed. Eight days after inoculation the temperature was 102°, but the following day it was normal. A blood culture proved negative.

On the sixteenth day the temperature again registered 102°,

and a blood culture proved negative. On the nineteenth day the temperature was 103.4° , and a blood culture again proved negative, and, although the temperature remained above normal up to the twenty-sixth day, the animal appeared perfectly normal and blood cultures taken on three other occasions all proved negative.

Bovine 2538 was inoculated with 1 c.c. of pleuro-pneumonia culture of a second generation and showed the first swelling over the site of inoculation on the seventh day. The temperature up to this period was normal.

The first jugular culture was made on the day the swelling was first noticed, *i.e.*, on the eighth day after inoculation, and cultures were made on the three following days.

The swelling remained stationary for a few days and then gradually subsided, and the animal was discharged thirty days after inoculation.

The temperature during the whole period remained normal. The culture tubes inoculated at various periods with blood from the jugular were all sterile.

Bovine 2213 was inoculated with 2 c.c. of pleuro-pneumonia culture, original strain and fourteen days old, and from the fifth day onwards cultures from the blood were made daily up to the fourteenth day.

On the sixth day following inoculation a faint thickening was observed near the site of inoculation. The temperature of the animal was normal. On the fourteenth day after inoculation the swelling had increased to 6-in. by 3-in., hard but not painful, and the temperature still remained normal.

The swelling began to subside, and the animal was discharged on the thirty-first day after the original inoculation. The blood cultures all proved to be sterile. Each culture was incubated for a period of fifteen days before it was discarded.

DISCUSSION.

The foregoing experiments have shown that there is no difficulty in obtaining a pure and virulent culture of the virus from the blood stream of artificially infected animals, and the virus can generally be demonstrated in the blood stream on the first day of a thermal reaction accompanied by a well-marked local reaction. The organisms remain in the blood stream until such time as the local reactions begin to subside and there is a corresponding drop of temperature.

In the case of *Bovine 2449* (page 173), which had been inoculated behind the shoulder, the last positive culture was obtained nineteen days after the original infection by inoculation although in this case the temperature remained above normal for the following two days and then began to drop. The local reaction had been gradually subsiding and the animal to all appearances looked as if it would make a recovery.

The ox was eventually discharged thirty-seven days after it

had been inoculated and all subsequent cultures from the nineteenth day were negative.

In cases when animals have succumbed or have been destroyed when *in extremis* as a result of an artificial infection the virus has been demonstrated in the blood up to the time of death.

There may be a temporary local reaction as a result of a subcutaneous inoculation of the virus without a corresponding thermal reaction, but during this period the virus has not been demonstrated in the blood stream, and this has often been observed when an animal is strongly resistant to the infection.

In one case in which a well-marked local reaction developed seven days after infection and the temperature was normal a pure culture was obtained from the blood, but the following day the temperature was above normal and a pure culture was again obtained.

The thermal reaction appears to denote the period when the virus may first be demonstrated in the blood, and on its severity depends whether an infection of the blood stream takes place and persists. Never have we yet seen a case where the local reaction has subsided and the blood stream remained infective, nor has there been a case in which the animal has succumbed to the effect of the inoculation and the blood stream been non-infective.

The comet-like bodies observed in the culture from Bovine 2039 were not observed in any other case, and one is inclined to surmise that they were clumps of the organism of pleuro-pneumonia, for the remaining portion of the gelatinous medium was perfectly clear and showed no signs of growth.

The cause of this clumping and of the gelatinisation of the whole medium except the lower portion is difficult to explain, especially as it was only seen in the first tube which was inoculated with the blood. These bodies being so numerous and minute, it was not possible to fish out any single clump for examination, and when the tube was shaken there was a tendency for the bodies to adhere together and the jelly-like medium was broken down.

It is therefore obvious that the organism is not distributed to other organs of the body for some time after an artificial inoculation of virulent culture.

Bovine 2628 was inoculated subcutaneously behind the shoulder with 2 c.c. of a P.P. culture of an original strain and was killed on the second day after a swelling appeared at the site of inoculation, which was the eighth day after inoculation, when the temperature was normal. The heart blood, spleen pulp, and pulp of prescapular and precrural lymphatic glands were inoculated into Martin's bouillon plus ox serum. The medium proved sterile after an incubation of fifteen days.

From the number of positive cultures obtained from the various organs of an animal which has died as a result of an artificial infection, it would seem that the blood stream plays no small part in carrying

the virus to the various organs, for the virus was isolated from most organs in the body and, according to the richness of the virus, a corresponding quickness of growth resulted in the inoculated media, the glandular organs apparently being particularly rich in virus.

IV.—THE INFECTIVITY OF THE BLOOD IN NATURAL INFECTIONS OF PLEURO-PNEUMONIA AND BLOOD CULTURES AS A MEANS OF DIAGNOSIS.

It was possible later in the year to continue the above work amongst naturally infected cattle. A herd consisting of 300 head revealed many clinical cases which were confirmed by slaughter.

Blood cultures were taken from a great many head of both obviously infected and suspicious cases and some in-contacts.

Early in the outbreak blood cultures were made just before the animal was slaughtered and there was then no opportunity to repeat the culture in the event of the first being contaminated, which unfortunately was the case in several instances.

Blood Culture Results.

Number of animals from which blood cultures were made before slaughter, 45.

Number giving contaminated culture only, eight. All these slaughtered immediately after the blood was taken. Post-mortem positive P.P.

Number giving positive culture, 14. At post-mortem, all were positive. Two were "lungers."

Number giving sterile cultures, 23. At post-mortem, 15 of these showed no signs of P.P., and eight were positive. All were "lungers."

Method of Making Cultures.

The animal to be bled was thrown in a sheltered spot and an area over the jugular vein was shaved and painted with iodine. A cord was fastened tightly round the neck and the vein thus distended. With a sterile syringe and needle the vein was pierced and a few c.c. of blood extracted, of which a few drops were immediately transferred, before coagulating, into the tubes of media over a flame.

Culture tubes were incubated at 37° C. and, if positive, sub-cultures were made to confirm the diagnosis.

Negative cultures were examined for fifteen days before being discarded, and where any suspicion of opalescence occurred a sub-culture was made in the hope that a very weak growth might be stimulated, but only once was a strong positive culture thus obtained from a poor original growth.

As in artificial infections of pleuro-pneumonia, so in a natural infection, the blood contains the causal organism and pure cultures may be obtained from it.

Of 45 animals whose blood was cultured eight animals gave a contaminated culture, and as each animal was slaughtered immediately after the blood was taken no second opportunity was afforded of making cultures. All were marked clinical cases of the disease

and all were positive cases on post-mortem. Of the remaining 37 animals 15 were found to be free of P.P. on slaughter and blood cultures were always negative.

The details of 22 cases all of which were positive on post-mortem remain to be examined. Of these, 14 gave positive cultures, and at least 11 showed no symptoms of the disease and the other three were only suspicious.

Two of this group were "lungers."

Thus eight out of 22 animals that proved positive by post-mortem were undetected by blood examinations, *i.e.*, about 33 per cent. All of these were "lungers."

This word "lungers" has been used for cases in which part of the solid lung has undergone necrosis and the dead portion has been encapsulated.

Such sequestered tissue is said to remain infected and animals carrying such a lesion are believed to be capable of spreading the disease.

Indeed a "lunger" has been looked upon as the cause of all new outbreaks, and, owing to the inability to detect the animal clinically, it remains the reservoir and carrier of the disease.

It will be noticed that of the eight animals not detected in the above outbreak all were "lungers," while two that might also be placed in this category were detected by blood culture.

It has been shown that an animal in the acute stages of pleuropneumonia is also in a state of septicæmia and blood culture with careful technique will give pure cultures.

Without being emphatic, one is inclined to the view that this state of septicæmia occurs late in the course of the infection—probably too late to be of any more value than definitely confirming a suspicious clinical diagnosis, although we have by blood cultures detected cases, later confirmed by post-mortem, that had passed undetected when clinically examined.

As stated above, however, sequestration may take place, in which case the organisms are usually no longer circulating and the blood becomes sterile.

This has not been confirmed, for in no natural case has an animal giving a positive culture been allowed to recover; but, whereas spleen cultures made from eleven positive acute cases gave eleven positive results, of those made from six "lungers" whose blood was negative all were sterile. In two cases sequestration was not complete and both gave positive blood cultures and one a positive spleen culture, the other not being tested.

During examination of the above-mentioned encapsulated lesions a striking feature was the very degenerated and caseous state of the sequestrum, and, while portions showed a resemblance to the pathological histology of lung in this disease, it was difficult to believe that any trace of virus could remain virulent in such a degenerated mass.

To test this, on three separate occasions part of such a sequestrum,

including the fibrous wall, was emulsified and filtered through a Seitz filter and in each case only sterile cultures were obtained, while control filtrations of known P.P. virus alone and P.P. cultures contaminated with other organisms always gave pure cultures.

Thus these sequestra were not virulent at the time of the animals' death and it is unlikely that they ever would have begun again. It would be of interest to know whether the majority of such encapsulated lesions are really non-infective, in which case a recovered animal need not necessarily be a carrier.

SUMMARY AND CONCLUSIONS.

Animals Infected by Inoculation.

The organism of Bovine Pleuro-pneumonia can be cultivated from the blood of an animal artificially infected.

The blood of an artificially infected animal when inoculated into a susceptible animal can produce a typical artificial infection, with lesions appearing after the same number of days as when a susceptible animal is inoculated with a pure culture of the organism.

The organism as a rule cannot be obtained from the blood at the time of a well marked local reaction without a corresponding thermal reaction. In most cases it can be obtained on the first and subsequent days of a thermal reaction accompanied by a local reaction.

The virus was obtained in pure culture from the spleen and prescapular glands and from nearly every organ of an animal that had died of an artificial infection.

The glandular organs appear to be most rich in virus.

Naturally Infected Animals.

Pure cultures of the virus of Contagious Bovine Pleuro-pneumonia can be obtained from the blood.

The blood is infected up to the period when the lung lesion becomes encapsuled.

The organism could not be recovered from lung sequestra in an advanced stage of degeneration.

With the aid of blood cultures cases of the disease have been detected when a clinical examination had failed.

Blood cultures offer a simple method of detecting and confirming a double clinical examination.

In the very early stages of infection, before the blood stream becomes infected, blood cultures are valueless as an aid to diagnosis.

From the spleen and glands of an infected animal pure cultures of the virus can be obtained.

The virus was not obtained from the spleen and glands of animals with encapsuled lung sequestra.

We wish to take this opportunity to acknowledge the assistance of Mr. J. Ford, Senior Laboratory Assistant, and Mr. W. H. Heynes, Laboratory Assistant, without whose help and interest in this investigation it would not have been possible to undertake all the blood cultural tests at a time when we were short-staffed and pressed with other duties.

GAS GANGRENE INFECTIONS OF SHEEP.

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IN a former paper dealing with a disease of sheep on the Romney Marsh termed "struck," reference was made to a condition popularly termed "gangrene" or "ganger" which may affect the ewe a day or two after lambing or the lamb a day or two after castration or docking, and occasionally appears in a flock of sheep soon after shearing. There is, however, always a clear history of wound infection. (McEwen and Roberts, 1931).

The infectious nature of the disease is recognised by farmers and shepherds, but in so far as the bacteriology of the condition is concerned no investigations appear to have been made until one of us (A.D.M.) in 1928-1929 studied material received from a number of cases and also had the opportunity to make a post-mortem and bacteriological examination upon a ewe a few minutes after death. *B. chauvoei* and *V. septique* were the pathogenic micro-organisms isolated at these examinations. The results of the investigations were in agreement with those of researches upon similar diseases in other countries, notably Germany.

During the springs of 1930-31 further cases were investigated, and in nearly all instances the post-mortem examinations and the collection of the material for bacteriological examination were made personally.

Certain fields on the Romney Marsh are reputed to be particularly dangerous for the parturient ewe, but there is not the same feeling regarding the relationship to the pasture where the disease occurs after docking, castration, or shearing. The disease occurs with greater regularity in the ewe after lambing and is then of greater economic importance. The possibility that it is transmitted by the hands of the shepherd and his lambing equipment is recognised because the majority of ewes succumbing to the disease have received assistance at lambing. The incidence of the disease, therefore, may be affected by factors quite irrespective of the degree of infection of the ground upon which the animals are grazing. One owner, whose observations we respect, states that when the disease assumes alarming proportions it may be stopped by moving all the pregnant animals to fresh ground and not permitting the shepherd to go near them, allowing the ewes to lamb by themselves if the services of a fresh shepherd are not available. It is only where the disease is assuming alarming proportions that such drastic methods would be resorted to. The disease occurs only during the latter half of the lambing season. It has not been possible to obtain figures of the percentage of losses caused by it. On one farm in 1930 thirty ewes were lost out of 300, but this proportion is in great excess of the

mortality over the whole area of the Romney Marsh. It is possible that the losses fluctuate between 2 and 3 per cent. The mortality among other classes of animals from a wound gas gangrene is of less economic importance, though when a considerable number of deaths occur simultaneously they cause alarm.

GAS GANGRENE IN THE PARTURIENT EWE.

Clinical Features.

Symptoms may become apparent at any time from 6 to 22 hours after lambing, but they are seldom noticed until 24 to 36 hours have elapsed. The animal ceases to feed and ruminate, remains apart from the flock, and neglects its lamb. Breathing becomes accelerated and the vulva and surrounding tissues tumefied and bluish-red in colour. The tumefaction and discolouration spreads and involves the whole perineal region, extending from the under surface of the tail above to the udder below. Drops of blood-tinged serous fluid exude through the skin. The respirations are laboured. After this stage is reached the ewe is no longer capable of standing, coma supervenes, and death quickly follows. The whole clinical course of the disease does not exceed 48 hours and in the majority of cases it is much shorter, death occurring on the third day after lambing.

Post-mortem Appearances.

A characteristic feature of the post-mortem examination is that lesions are chiefly confined to the perineal region. The skin of these parts is discoloured, varying from a pink to a purplish tint. Beads of pink serous fluid may exude through the skin. The wool over the affected area is easily detached and may be found matted in little patches with this serous exudate. Palpation of the affected region reveals underlying œdema and tumefaction but not crepitation, and on incision the subcutaneous and intermuscular tissues are found infiltrated with a large amount of gelatinous liquid, pink to purple in colour. Gas is absent from these infiltrations. The muscular tissues of the affected parts may show slight tumefaction and darkening but these lesions are not constant. There are cases, however, where the musculature is extensively involved and the muscles of the loins and of the thighs swollen and very dark in colour, and in some instances portions of the affected muscle are emphysematous, presenting the typical blackquarter appearance. The sourish smell associated with blackquarter is occasionally noted. The mucosa of the vagina generally shows areas of erosion and necrosis, these occurring most regularly in the neighbourhood of the fornix. The cervix of the uterus may show similar lesions and its folds be œdematous. The uterus in its gross appearance is generally comparable with a healthy organ examined at a similar period after parturition, but its walls and the broad ligament may be markedly œdematous and thickened. Occasionally a moderate excess of peritoneal fluid is encountered. This fluid is sometimes slightly blood-tinged.

When the animal has been dead for some hours the subcutaneous tissues over the whole body or the greater part thereof are puffy from distension with gas and a moderate quantity of semi-fluid material. The skeletal muscles of the body are dark, moist, and friable and the intermuscular septæ contain gas and gelatinous fluid. Changes of a putrefactive nature are advanced in the abdominal and thoracic cavities, both of which contain an excess of blood-tinged fluid, and there is hæmoglobin staining of the viscera, imparting to them a false inflammatory appearance.

WOUND GAS GANGRENE OF LAMBS.

Animals generally die on the second or third day following the operation of castration and docking. Clinically they do not show any features which might not be expected from an acute wound infection and a septicæmic condition. Few lesions were encountered in lambs. The subcutaneous tissues were moist and the thigh muscles slightly darker in colour than normal. No lesions were found in any of the internal organs.

We have not had an opportunity to examine a case of gas gangrene following shearing, but from the reports given by shepherds the disease appears to be similar to that encountered in lambs.

BACTERIOLOGY.

The bacteriological methods employed during the 1928-29 seasons consisted in isolating pure strains of bacteria from specimens of muscle sent to the laboratory by veterinary surgeons, farmers, and shepherds, and in identifying them.

The identification of the bacteria was based upon the type of colonies produced on serum agar surface slants, in shake liver agar, and shake blood agar; the cultural reaction in broth containing minced meat, nutrient broth, alkaline egg broth, milk media, milk containing a portion of brain tissue, broth containing brain tissue and a piece of iron wire, broth containing a cube of coagulated egg albumen; and fermentation reactions, glucose, lactose, saccharose, salicin, inulin, glycerine, and mannite being used as the test substances.

During the season the only pathogenic anærobes encountered were *B. chauvæi* and *V. septique*. These two micro-organisms were distinguished one from another by the following reactions:—

	<i>B. chauvæi.</i>	<i>V. septique.</i>
Surface colonies ...	Discrete, round or slightly irregular borders.	Spreading, with long filamentous processes.
Shake agar ...	No growth ...	Large woolly colonies.
Shake blood agar ...	Pinpoint compound colonies.	Large woolly colonies.
Milk ...	No growth ...	Good growth, clot and gas.
Milk and brain tissue ...	Large smooth clot, small volume of gas.	Stormy clot, large volume of gas.
Saccharose ...	Fermented ...	Not fermented.
Salicin ...	Not fermented ...	Fermented.

During the 1930 season the strains of pathogenic anærobes isolated were studied in a similar manner except that the blood agar medium was omitted.

Each strain as it was isolated was inoculated into guinea-pigs, and the characteristic lesion for *B. chauvæi* and *V. septicæ* infection were reproduced in every instance when these respective micro-organisms were inoculated.

In the 1931 season we did not consider it necessary to undertake detailed cultural examinations with a variety of media in order to establish the identity of the strains, and in the case of *B. chauvæi*, examinations were confined to the character of the growth and its microscopical appearance in minced medium and on serum agar slants. The *V. septicæ* strains were studied in the same two media, and their fermentation reactions were also ascertained. Again all strains were inoculated into guinea-pigs, the lesions were noted, and smears made from the seat of inoculation and peritoneal surface of the liver were examined. Further, with the *B. chauvæi* strains isolated in 1930 and 1931 identification of the micro-organism was confirmed by the neutralisation of the pathogenic action of the cultures by small quantities of specific immune serum, guinea-pigs being used as the test animals and suitable controls included for every strain tested. The immune serum was prepared by the repeated inoculation of a sheep with large quantities of *B. chauvæi* (ovine strain) anaculture.

The *B. chauvæi* strains when first isolated were all very pathogenic, 0·1 c.c. killing guinea-pigs in 16 to 24 hours. With storage and sub-cultivation there was a considerable loss of virulence. All the *V. septicæ* strains were highly pathogenic for guinea-pigs, and there has been no suggestion of their having become any less virulent by storage or sub-cultivation.

In only one instance has a pathogenic anærobic bacillus other than *B. chauvæi* and *V. septicæ* been encountered in these investigations. This was isolated in pure culture from the pale subcutaneous fluid and also from the muscles around the vagina of a ewe which had died three days after lambing. The organism gave the characteristic reaction for *B. œdematiens* in culture. It produced the typical pale œdematous lesions on inoculation into guinea-pigs. Further, its pathogenic action was neutralised by *B. œdematiens* serum. It is regarded as a typical strain of *B. œdematiens*. This case was particularly interesting in that the appearance of the lesions in the ewe was suggestive of a *B. œdematiens* infection. The lesions differed from those encountered on all the other occasions in the greater œdema of the region of the vulva and in the palor of the skin of the affected parts. Again, the infiltrating fluid was a pale straw colour, the subcutaneous vessels were injected, and the musculature was paler than in normal animals.

During the preliminary examinations conducted in 1928-29 non-pathogenic bacteria had been encountered on a number of

TABLE

Case.	Time between Parturition and Death.	History of Lambing.	Time between Death and the Examination.	Distribution of Lesions.	Smear Examinations of Local Lesions.	Materials from which Cultures were made.	Bacteria Isolated.
1	3 days	normal	15 minutes	Vagina, cervix, walls of uterus and broad ligament, subcutis of perineum and tail	B. chauvœi type	Muscle of thigh and subcutaneous tissues of tail, heart blood, and spleen	B. chauvœi
2	2-3 days	Assisted	Immediately	Vulva and perineum	B. chauvœi type	Local lesion, heart blood, and peritoneal fluid	B. chauvœi
3	2-3 days	—	Killed and examined immediately	Perineum, vulva, and muscles of thigh	B. chauvœi type	Subcutaneous tissues of vulva, muscle, and peritoneal fluid	B. chauvœi
4	3 days	—	Immediately	Perineum, vulva, and muscles of thigh showed typical blackquarter tumefaction	B. chauvœi type	Muscle and heart blood	B. chauvœi
5	24 hours	Normal	Immediately	Vulva and perineum, purulent condition of mammary gland	B. chauvœi type ; mammary gland, a short, stout Gram-positive bacillus	Muscle and subcutaneous tissue of vulva	B. chauvœi
6	4 days	—	Killed and examined immediately	Vulva and perineum. Moderate inflammation of the small intestine	Cocci	Local lesion, heart blood, spleen, and peritoneal fluid	Staphylococcus
7	2 days	Normal	1 hour	Perineum, cervix of uterus, muscles of thigh	B. chauvœi type	Muscle and heart blood	B. chauvœi
8	2 days	Assisted	2 hours	Vulva, perineum, and muscles of thigh	B. chauvœi type	Subcutaneous tissues of vulva and muscle	B. chauvœi

9	2 days	Assisted	7 hours	Perineum, vagina, cervix of uterus	B. chauvœi type	Subcutaneous tissues of local lesion and muscles of thigh	B. chauvœi
10	2-3 days	—	7 hours	Vulva, perineum and tail; œdema of uterus	B. chauvœi type	Subcutaneous tissues of local lesion	B. chauvœi
11	—	—	7 hours	Vulva, perineum, and tail	B. chauvœi type	Subcutaneous tissues of local lesion and thigh muscle	B. chauvœi
12	Exact time not known, probably 3-4 days	—	6-10 hours	Carcass had been skinned and eviscerated by shepherd. When examined the thigh muscles showed extensive lesions	B. chauvœi type	Muscle of thigh	B. chauvœi
13	3 days	Normal	6-10 hours	Perineal region, vulva, lumbar and thigh muscles	B. chauvœi type	Muscle	B. chauvœi
14	2-3 days	Assisted	Found dead in morning	Vulva and perineum	B. chauvœi type	Local lesion	B. chauvœi
15	3 days	—	Found dead in morning	Vulva and perineum	B. chauvœi type	Local lesion	B. chauvœi and V. septicæ
16	2 days	—	Found dead in morning	Vulva and perineal region. Underlying muscles very pale	An anaerobic type of bacillus not similar to B. chauvœi	Subcutaneous tissues of vulva and muscle	B. œdematiens
17	24 hours	Assisted	Found dead in morning	Perineum and vulva	B. chauvœi type	Local lesion	V. septicæ and another anaerobic bacillus not identified
18	3 days	Assisted	Found dead in morning	Vulva, perineum, and vagina	Considered of V. septicæ type	Local lesion	V. septicæ
19	2-3 days	—	Found dead in morning	Vulva and perineum	B. chauvœi type	Local lesion	B. chauvœi
20	2-3 days	—	24 hours	Vulva and perineum	B. chauvœi type	Local lesion	V. septicæ

occasions, but these were considered as in all probability contaminants and discarded. The results of the last two seasons' examinations appear to justify this assumption, as when the material was fresh the invading micro-organism was present in a pure state.

Details of the history of the case were often omitted by the senders of the material in the 1928-29 investigations, and it is possible that some of these specimens came from sheep which had died from some disease other than gas gangrene. In five instances, however, it was definitely stated that the ewe had died from "gangrene." From three of these *V. septique* was recovered, and from the remaining two *B. chauvæi*. Twenty-eight specimens received without a history showed *V. septique* in 18 instances and *B. chauvæi* in ten. In our researches on the disease "struck" we have never recovered *B. chauvæi* from the carcase of affected animals even after these had been lying on the field several hours, but *V. septique* was found on several occasions in carcasses which were examined some hours after death and it was regarded as a post-mortem invader (McEwen and Roberts, 1931). It is therefore improbable that the ten cases from which *B. chauvæi* was isolated were anything but cases of gangrene, though doubt may be entertained regarding the nature of the disease in the 18 instances where *V. septique* was isolated. These results indicated that *B. chauvæi* and *V. septique* were both capable of causing the disease. In 1930-31 *B. chauvæi* was encountered more frequently than *V. septique* and the latter was never found invading the tissues of sheep when these were examined at or very soon after death.

The results of the examinations made upon material collected personally, and where the interval elapsing after death was known, are recorded in the Table.

The table reveals the relationship between the species of bacterium isolated and the time which elapsed between death and the bacteriological examination. Thus, 13 cases examined within 10 hours of death all gave cultures of *B. chauvæi*, this being the only pathogenic bacterium isolated, and in all but one instance it was found in the local lesion in a pure state. In six cases the exact time of death was not known, since the sheep had died in the night and material was collected the following day, but the interval would be about 10-16 hours. In two of these cases *B. chauvæi* was the only micro-organism isolated; in one instance both *B. chauvæi* and *V. septique* were recovered; in two cases *V. septique* alone was isolated, and in the sixth case *B. œdematiens* was found to be the invading micro-organism. One case examined at the time of death showed a pure staphylococcal infection.

The preponderance of *B. chauvæi* over *V. septique* is illustrated in these investigations where material was collected personally, and this fact, coupled with the striking absence of *V. septique* in fresh cases, raises the query as to whether *V. septique* was anything more than a post-mortem invader.

During the 1929 season material from five lambs said to have died from "gangrene" following upon castration was examined, and in each case *B. chauvæi* was the only pathogenic bacterium isolated. In 1930 four similar cases were obtained personally, and in each case *B. chauvæi* was found in the muscle of the hind limbs in abundant and pure culture.

GAS GANGRENE IN SHEEP FOLLOWING UPON VACCINATION.

During the past season, 1931, experimental vaccination of approximately 1,500 sheep was carried out against "struck" with an anatoxin prepared from bacteria-free filtrate of cultures of *B. paludis*. On all but one farm these inoculations had been made personally, the sheep having been held in a standing position and inoculated into the subcutaneous tissues at the back of the shoulders after the skin had been cleaned with methylated spirits containing carbolic acid or with weak tincture of iodine. No ill effects followed these vaccinations, great care having been taken to prevent, as far as possible, contamination of the hands with soil and to keep the vaccine and instruments used in making the inoculations sterile. On one farm, however, vaccinations were not made personally and 120 ewes were inoculated in the thigh, the animals being caught and held in a very muddy pound; 15 per cent. of these sheep died from gas gangrene as a direct result of the inoculation. Five of these animals were examined and four showed a pure infection with *B. chauvæi*. With the fifth there was a mixed infection, but neither *B. chauvæi*, *V. septique*, nor any recognised species of pathogenic anærobe was isolated. This illustrates very clearly the heavy contamination of the soil on the Romney Marsh and the extreme susceptibility of sheep to infection with *B. chauvæi*, and from our observations it would appear that under natural conditions sheep are more susceptible to *B. chauvæi* than to infection with *V. septique* or any other species of bacterium of the gas gangrene group.

It is surprising that spontaneous cases of blackquarter analogous to the bovine disease are not of frequent occurrence. Cave (1907), in his researches on "struck," states that that disease is in all respects a blackquarter caused by the blackquarter bacillus, but from his description of the bacillus it would appear that he was not dealing with *B. chauvæi* but with a micro-organism "stout and with rounded ends," which more closely resembles *B. paludis*. In the course of examinations upon an extensive number of sheep which have died suddenly or after a short illness, and without a history of wounding, we have recovered *B. chauvæi* on one occasion only. This animal was a two-year-old ewe, and it had been observed to be ill only a few hours before death and had been eviscerated and hung up by the owner at the time of death. It was examined 12 hours after death, and the left thigh showed a typical blackquarter lesion; no other abnormalities were found.

It is interesting to note that on this farm, though on a different field, blackquarter had appeared in a group of 20 recently purchased

eighteen-months-old heifers and six had succumbed to the disease. We obtained a strain of *B. chauvœi* from one of these heifers which had died a day or two after removal from the Marsh pasture; culturally it was indistinguishable from the ovine strains, and *B. chauvœi* anti-serum produced with an ovine strain protected guinea-pigs against this bovine strain.

DISCUSSION.

When the results of the bacteriological examinations of all the material received are considered independently of the history of the cases, the two micro-organisms which appear to have ætiological significance are *B. chauvœi* and *V. septique*. This is in substantial agreement with the findings of Miessner and Albrectht 1924, Ræbiger and Speigl 1924, Manniger 1924, Knall 1924, and Wolters 1927, all these authors attributing significance to *V. septique* though agreeing that the majority of the cases of "gas œdema" in sheep are caused by *B. chauvœi*.

Miessner and Albrecht examined 56 specimens of muscle sent to the Veterinary High School at Hanover. From 54·28 per cent. *B. chauvœi* were isolated, 18·7 per cent. showed *V. septique*, and 18·7 per cent. *B. chauvœi* and *V. septique*, 8·42 per cent. gave *B. welchii* or *B. welchii* with *B. chauvœi* or *V. septique*. In eight instances no bacteria were isolated. Ræbiger and Speigl report that 48 to 94 per cent. of cases are due to *B. chauvœi* and 6 to 52 per cent. to *V. septique*. Manniger, in a flock where death followed castration, isolated *B. chauvœi* from the cases he examined; on the same farm eleven sheep died after shearing and *B. chauvœi* was isolated and regarded as the causal micro-organism, but in another flock, where the disease followed parturition, *V. septique* was recovered. Knall reports the isolation of *B. chauvœi* from 22 cases, *B. chauvœi* and *V. septique* from two cases, *B. chauvœi*, *V. septique* and another micro-organism from two cases, and in one instance *V. septique*. Wolters studied 44 cases, and in 30 he found a *B. chauvœi* infection, but in thirteen *V. septique* was recovered and in one *B. sporogenes*. The greater importance of *B. chauvœi* is further emphasised by the findings of Marsh 1919 and 1923, and of Marsh, Welsh, and Jungherr 1928. The former author reported two outbreaks of gas gangrene in sheep due to *B. chauvœi*, and Marsh, Welsh, and Jungherr recorded sixteen outbreaks; a bacteriological examination was made upon one sheep from each of thirteen of these outbreaks and *B. chauvœi* was isolated in ten instances.

If it were possible to attribute ætiological significance to any pathogenic anærobe found in the tissues at the time of examination, then the view that *V. septique* is the cause of the disease in a number of instances could be accepted. But when consideration is given only to those cases which we have examined and where the history is known, it is found that in no instance was *V. septique* recovered when the bacteriological examination was made at the time of

death or very soon after death, and it was only after a considerable lapse of time that *V. septique* showed itself.

In our report on our investigations upon "struck" we recorded the isolation of *V. septique* from a number of cases examined some time after death and under circumstances where it could be regarded as a post-mortem invader of the carcase. This conclusion was justifiable because the characteristic lesions of "struck" are produced by the toxin of *B. paludis* and so permit the ruling out of *V. septique* as the cause of the disease in the few instances where it was recovered. The same definite conclusions regarding *V. septique* are not applicable in cases of gas gangrene on account of the close similarity between the lesions produced by *B. chauvæi* and *V. septique* in the subcutaneous and muscular tissues when these micro-organisms are experimentally inoculated into sheep. However, should *V. septique* appear as a post-mortem invader in the disease "struck" it is likely to play a similar part in other diseases of sheep grazing upon the same ground; and should the initial lesion of gas gangrene be due to *B. chauvæi*, but *V. septique* be present in the lesion as a post-mortem invader, it is probable that the latter organism will obscure the presence of the former at bacteriological examination. Microscopically they frequently cannot be distinguished with any degree of certainty, and in culture *V. septique* would rapidly outgrow *B. chauvæi*, making it impossible to detect the presence of that organism. We have tried to separate *B. chauvæi* from *V. septique* by cultural means when they were present in a mixture in varying proportions ranging from one part of *B. chauvæi* and one part of *V. septique* to 1,280 parts of *B. chauvæi* and one of *V. septique*. These mixed cultures when sown on serum agar slants invariably produced a surface growth of *V. septique* which obscured any developing *B. chauvæi* colonies; in minced meat medium the cultures morphologically resembled *V. septique* rather than *B. chauvæi*, and sowing from the minced meat medium cultures to serum agar slants resulted in the production of a spreading growth of *V. septique*. We consider that in many instances a *B. chauvæi* infection would be completely masked were there even a moderate post-mortem invasion of the part with *V. septique*, and in those rare cases when *V. septique* and *B. chauvæi* have both been isolated that the former bacterium was probably present in very small numbers and may have been present in one lot of seed material and not in another. Therefore, *V. septique* cannot definitely be incriminated as the cause of gas gangrene in sheep until it is isolated from perfectly fresh material.

The mere ubiquity of highly pathogenic anærobic bacteria—micro-organisms which on parenteral inoculation of the sheep are as pathogenic as *B. chauvæi*—does not necessarily mean that the micro-organisms will be found to cause natural cases of gas gangrene in these animals. Cases of gas gangrene and "struck" occur on the same pastures and at the same time of the year. The contamination of these pastures by *B. paludis* must in many instances be heavy,

and the hands of the shepherd are at times no doubt contaminated by *B. paludis* while he attends his ewes at lambing, yet we have not found gas gangrene infection to be caused by this organism or by any bacteria of the *B. welchii* type. Again, the frequent recovery of *V. septique* indicates that the organism is very widely distributed on the pastures, but there is no conclusive evidence that it causes the disease. A case of particular interest was the one in which a typical strain of *B. œdematiens* was isolated. The case unfortunately was not obtained at the time of death, but from the appearance of the local lesions it is considered that *B. œdematiens* was the causal micro-organism.

SUMMARY.

A bacteriological examination has been made of 68 cases of gas gangrene, a disease which causes serious mortality among sheep on the Romney Marsh.

The disease is a common one in ewes shortly after lambing, the infection gaining entrance to the tissues through abrasions or wounds of the genital organs. *B. chauvœi* is usually the cause.

In gas gangrene in lambs the infection gains entrance to the body through wounds inflicted at castration or docking. Nine cases have been studied and *B. chauvœi* was recovered in all of them.

Gas gangrene has been found following vaccination with sterile material when the vaccination was done under adverse conditions and there was a great risk of contamination from the soil. Five of these cases were examined and *B. chauvœi* was the causal micro-organism in four cases. The fifth case showed a mixed bacterial infection, but no recognised species of bacterium of the gas gangrene group was recovered.

The soil of the Romney Marsh appears to be heavily contaminated by *B. chauvœi*, *V. septique*, and *B. paludis*.

B. paludis or bacilli of the *B. welchii* type have not been encountered in cases of gas gangrene. *B. œdematiens* appeared to be responsible in one case.

V. septique has not been proved to be the cause of gas gangrene, and it has not been definitely incriminated in any of our investigations into sheep diseases. It is frequently recoverable, apparently as a post-mortem invader, from the bodies of animals that have been dead for some time.

Despite the wide distribution of *B. chauvœi* throughout the Romney Marsh, only one case has been encountered in which it is probable that the infection did not occur through a wound but spontaneously in the manner of blackquarter in the bovine.

Prophylactic measures should be directed primarily against a *B. chauvœi* infection.

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DISEASES OF GOATS IN NIGERIA.**II.—NOTES ON A FATAL SEPTICÆMIC INFECTION.**

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INTRODUCTION.

DURING the investigations in Nigeria a particularly virulent condition was produced in goats by the subcutaneous inoculation of blood from a goat that showed a high thermal reaction and died five days later from Contagious Pleuro-pneumonia.

This condition was passed with ease from goat to goat by inoculation of blood during the thermal reaction, but blood filtrates were negative.

For some time cultures proved sterile or contaminated but later, on special media and allowing 6-8 days for a growth, a pure culture of a very small micro-organism was isolated from the jugular vein of a goat at the height of the reaction.

Inoculation of this organism subcutaneously produced a similar condition to that by blood inoculation and the organism could be isolated from the heart-blood and spleen.

It was believed that this organism was the causal agent of contagious pleuro-pneumonia in goats, as on intra-thoracic inoculation of culture or blood a croupous pneumonia was produced, not unlike that seen in that disease and from which the organism could also be isolated in pure culture.

Much work was therefore done on vaccination against this micro-organism with a view to noting at the first opportunity the immunity of vaccinated animals against a natural outbreak of contagious pleuro-pneumonia.

Unfortunately, however, during the writer's absence from the country all the cultures maintained here without passage in animals failed to grow. Cultures on solid media were taken to the Curator of type cultures at the Lister Institute, London, and to Mr. I. A. Galloway, B.Sc., M.R.C.V.S., of the National Institute for Medical Research, London, for identification and for work on its biology. Both these workers however reported complete cessation of growth after a few months only. In view of these similar results it is possible that the organism would not live for long "in vitro," as, prior to my absence from the laboratory, it was frequently passed through its presumed natural host, the goat, and new virulent cultures were isolated. Against this, however, it must be stated that one strain was taken to the 18th generation, of six or seven days each, representing at least 108 days out of the animal body, and it still remained equally virulent for goats.

Though experimentally this organism caused a rapidly fatal disease in goats, characterised by an acute swelling at the site of inoculation and *tumor splenis* on post-mortem, a natural disease simulating this was not known, though it was recognised that the

artificial condition was probably quite different from the natural disease, as is the case in Bovine Pleuro-pneumonia.

The work was summarised in the Annual Report of the Chief Veterinary Officer, Nigeria, in 1929, but it was hoped that the organism would be recovered later and further light thrown on the disease before publishing the results in detail. As this has not been realised, the work already done is considered of sufficient interest to report, the diseases of the goat now being the subject of closer investigation.

THE ORIGINAL TRANSMISSION.

Goat 81 died on November 14th, 1927, of typical Contagious Pleuro-pneumonia. Cultures of the pleural exudate gave a turbidity but no organisms were visible.

A second generation, also turbid when six days old, was inoculated subcutaneously and intra-pulmonarily into a series of goats, three of each.

Of these, one inoculated intra-pulmonarily died of a purulent pneumonia. All the others were negative except No. 110 inoculated subcutaneously, the subsequent history of which was as follows:—On the 14th day after injection the temperature rose to 105·6° F. and remained elevated several days, and the animal died on the 20th day from inoculation. Post-mortem showed typical pleuro-pneumonia but lung cultures were contaminated.

On the 16th day from inoculation, when the temperature was 105° F., this goat was bled to 10 c.c. from the jugular and Goat 115 was inoculated with this subcutaneously behind the shoulder. The temperature of this animal rose to 104·8° F. on fourth day, maximum 105·6° F. on sixth day, and it died on the ninth day with acute peritonitis and a subcutaneous œdematous swelling at the site of inoculation. On the eighth day of its reaction, when its temperature was 105° F., 10 c.c. of its blood in citrate solution was inoculated subcutaneously into Goat 119. Its temperature rose to 104·4° F. on the fourth day and reached a maximum of 105·4° F. on the fifth day, and death occurred on the tenth day. Blood cultures on the sixth day were apparently sterile when discarded after four days inoculation, but the blood subinoculated was virulent.

Post-mortem showed marked *tumor splenis* and acute local swelling.

The strain was continued in this way through more than seven sub-inoculations in goats before a pure blood culture was obtained in Martin's bouillon plus ox serum. Previous negative cultures on other media had led one to believe that the causal agent was ultra-visible.

FILTRATION OF BLOOD.

Blood from Goat 125—one of those used on the first series which gave a typical reaction—was passed through a Berkefeld

filter in a dilution of 2 per cent. Filtration took 10 minutes and cultures of filtrate were sterile 10 days later. Two goats, Nos. 141 and 144, were inoculated subcutaneously with 200 c.c. of this filtrate. Goat 138 was inoculated subcutaneously with 2 c.c. of the same blood unfiltered. Neither of the goats Nos. 141 and 144 reacted, but control goat 138 gave a temperature reaction and a local swelling. On the fifth day the temperature was 107.8° F. and the swelling extended all over the chest, along the abdomen and down the foreleg of the inoculated side. It was slaughtered *in extremis* on the same day. Heart blood cultures gave a slight opacity in two days and after five days a very fine micro-organism was just visible in stained smears of the culture.

This was considered to be the first pure culture obtained of the micro-organism in question. Both blood and blood culture from this case, subinoculated, produced a similar reaction to that seen previously.

The filtrate of the blood was therefore innocuous. Similar results were obtained by filtration of lymph from the large œdematous swelling which was always produced on subcutaneous inoculation of any virulent material. This lymph was virulent and a strain was maintained in this way for several successive passages.

SUMMARY OF INFECTIOUS TISSUES.

Blood in doses of 2 c.c. produced the typical condition; 0.02 c.c. was infective but the animal survived, only to succumb later to a larger dose.

Emulsion of spleen was used in doses of 2 c.c. to maintain a strain for 7 generations through goats.

When the lungs were pneumonic the pleural lymph was virulent, as was also the œdematous fluid produced by subcutaneous inoculation.

Pure culture in a dose of 0.005 c.c. was fatal, though the incubation period was increased to 14 days.

CONTACT.

An effort was made to transmit a natural infection to healthy goats by contact with inoculated animals.

May 10th, 1928.—Goat 275 was inoculated intra-pulmonarily with 0.5 c.c. of strain 142, 17th generation, five days old; and young goats 276 and 272 were placed in contact, all being kept in strict isolation.

Goat 275 died in five days after a temperature curve with a maximum of 105° F. There was pain at the site of inoculation and the animal limped on the inoculated side. On post-mortem there was present a slight broncho-pneumonia and swollen spleen. Pure cultures were isolated from the lung and the spleen.

May 17th, 1928.—Goat 118 was inoculated intra-pulmonarily with 0.5 c.c. of strain 142 of the 18th generation, five days old, and goats 276 and 272 remained in contact. The temperature of Goat 118 rose steadily to over 107° F. and the animal died on

the sixth day. It was lame on its right foreleg, breathing was laboured, and there was coughing. Post-mortem showed an acute croupous pneumonia and swollen spleen. Pure cultures of the micro-organism were isolated from the lung and the spleen. Goats 276 and 272 remained healthy. On the 32nd day each goat received 0.5 c.c. of virulent culture and both died, one in four days and one in eight days, each showing the typical symptoms. They were therefore susceptible but failed to become infected by contact with the artificial disease.

SUSCEPTIBILITY OF OTHER ANIMALS.

The blood of Goat 125 used in the blood filtration experiment mentioned above, taken at the height of temperature reaction (106.4° F.), was inoculated subcutaneously into sheep 44, ox 545, pigs 12-13, two guinea-pigs, and two white rats, in doses of 10 c.c., 10 c.c., 5 c.c., 2 c.c., and 1 c.c. respectively, and Goat 137 was used as control and given 10 c.c.

Ox 545 showed a very slight swelling which disappeared in two days, but without showing clinical disturbance, and the pigs, guinea-pigs, and rats were unaffected.

Sheep 44 had a temperature of 104.6° F. on the next day, with a maximum of 108° F. on the third day, and it died on the fifth day. There was an acute local œdematous swelling and a much enlarged spleen on post-mortem. Goat 84 was inoculated with 10 c.c. of this sheep's blood taken at death, and there was the usual clinical reaction with death on the third day and a much enlarged spleen on post-mortem. The passage through sheep had certainly not attenuated it for the goat. The control Goat 137 had a temperature of 104.6° F. on the next day, a maximum of 107° F. on the second day, and was dead on the fifth day, with a local swelling and an enlarged spleen.

Later, however, four sheep were inoculated, two with virulent blood and two with culture, and none of them reacted though control goats died. It is possible these sheep were not susceptible.

SYMPTOMS.

As already stated, blood, spleen emulsion, lymph from a local swelling, and pleural exudate or lung emulsion where pneumonia was present, are all equally virulent, and the result of inoculation at various sites as described below are similar for each type of inoculum. The same held true for pure cultures of the organism.

SUBCUTANEOUS INOCULATION.

The following, one of many similar reactions, may be taken as a typical case.

On *January 11th*, 1928, Goat 137 was inoculated with 10 c.c. citrated blood of Goat 125, bled on the sixth day when the temperature was 106° F. The site of inoculation was subcutaneously behind the shoulder.

January 12th, 1928.—Temperature 104·6° F., swelling just commencing.

January 13th, 1928.—Temperature 106·4° F., swelling extensive all round site of inoculation.

January 14th, 1928.—Temperature 107·0° F., swelling fairly tense, extending down forearm.

January 15th, 1928.—Temperature 107·0° F., great pain, animal unable to rise and not feeding.

January 16th, 1928. Dead.

INTRA-PERITONEAL INOCULATION.

January 6th, 1928.—Goat 133 inoculated intraperitoneally with 15 c.c. citrated blood of Goat 119 on sixth day of its reaction from blood passage and when temperature was 105·2 F.

January 7th, 1928.—Temperature 103·0° F., dull.

January 8th, 1928.—Temperature 104·2° F.

January 9th, 1928. 105·4° F.

January 10th, 1928.—Temperature 105·6° F.

January 11th, 1928.—106·9° F. No lesions at site of inoculation, respirations increased, great abdominal pain, diarrhoea.

January 12th, 1928.—Temperature 103·4° F., *in extremis*, slaughtered.

INTRAPULMONARY INOCULATION.

January 12th, 1928.—Goat 142 inoculated intrapulmonarily right side with 2 c.c. heart blood taken at post-mortem from Goat 133 above.

January 13th, 1928.—Temperature 102·6° F.

January 14th, 1928.—105·0° F., and remained about this until 18th, when reached 105·4° F.

January 19th, 1928.—Temperature 103·0° F. No local swelling, chest painful.

January 20th, 1928.—Temperature 102·0° F. Great pain over chest, slaughtered *in extremis*.

PATHOLOGICAL ANATOMY.

This varied somewhat according to the site of inoculation, but it was constant for all types of inoculum, including a pure culture of the micro-organism.

Subcutaneous Inoculation.—On the day following inoculation—a slight puffiness appears at the site, becoming more extensive each day. It involves the adjacent dependent portions and becomes œdematous and tense. If the foreleg be affected the animal is lame and unable to put its foot to the ground, and finally becomes completely recumbent, refusing food and water for at least 24 hours before death. There are the usual symptoms accompanying fever. Death takes place in from 3-8 days. On post-mortem the local lesion is characteristic and resembles very closely that produced in cattle inoculated subcutaneously with a culture of Contagious Bovine Pleuro-pneumonia.

There is marked hyperplasia of the local connective tissue—a fibrous “tumour” without the presence of blood vessels—and the tissue is infiltrated with straw-coloured lymph which tends to coagulate on exposure to the air. There is no encapsulation, but close adhesion to the skin and muscular tissue.

When the inoculation is behind the shoulder the ribs show acute congestion and the thoracic cavity may or may not give indications of commencing pleurisy.

Internally the one constant change is enlargement of the spleen.

This organ is tumefied and weighs from 200 to 400 grammes compared with the average normal of 80 grammes. The vessels of the capsule are congested and the parenchyma is darkened in colour and much softer in consistence, and the usual structures are not apparent, the whole being a homogeneous pulp. Occasionally a slight pneumonia may be present as described under the pathological anatomy of intra-pulmonary inoculation.

Intra-peritoneal Inoculation.—The site was usually on the right side of the abdomen just behind the last rib and about 3 inches below the vertebræ.

The subcutaneous tissues present a ring of slight congestion with some fibrous thickening at the point of entry of needle into the peritoneal cavity.

The omentum, the serous coat of the intestine, the capsule of the liver, kidneys, and spleen, are all covered with a fine film of white slightly tenacious pus. Several hundred c.c. of thin white fluid may be found free. The spleen is swollen as described above and usually adherent to the diaphragm and the rumen by white coagulated lymph. A small portion of the lung may be infected with the type of pneumonia to be referred to later.

Intrapulmonary Inoculation.—The site chosen for this type of inoculation was on the right side, two inches below the vertebræ, between the seventh and eighth ribs.

Subcutaneously there is usually a slight hyperplasia of the local connective tissue and a little congestion.

The thoracic cavity on the inoculated side, and occasionally extending to the other side, shows acute pleurisy of the whole wall, with fibrous adhesions. Varying amounts of free cloudy fluid are present. The inoculated lung is consolidated, the amount varying from a part the size of a golf-ball to three-quarters of the complete lung of that side. The centre of the consolidated area is of a slight grey colour with a ring of hyperæmia, *i.e.*, grey and red hepatisation. At times there are several of these areas of consolidation, and these evidently coalesce to involve a whole lobe or the whole lung.

No necrosis was ever seen as the subject usually dies before the condition is very advanced. There may or may not be an effusion of lymph on the pleura, but it is not so thick and much

paler than the typical yellow exudate seen in natural cases of contagious pleuro-pneumonia of goats.

THE MICRO-ORGANISM.

Culture Methods.—The first pure culture of the micro-organism involved in this condition was obtained from the heart blood of Goat 138, slaughtered *in extremis* and the autopsy made immediately. The goat had been inoculated subcutaneously five days previously with virulent blood, and it showed a huge swelling all around the site of inoculation. The temperature was 107·8° F. on the morning of its death.

Martin's bouillon to which 10 per cent. ox serum was added was inoculated with a drop or two of heart blood, which just clouded the medium. At 24 hours a clot was formed at the bottom of the tube and the medium was water clear; similar at 48 hours. At 72 hours, however, there was a suspicion of opalescence, only noticeable in comparison with a control tube, and this opalescence was more marked at four days. Never until after many generations, and then only after the fifth and sixth day, was the growth really recognisable without control tubes.

Beef broth, liver broth agar slopes, and glycerine agar, all failed to give a growth, but on ox serum agar and better still on inspissated serum, especially goat serum, very fine translucent, discrete, pin-point colonies were obtained after four or five days incubation.

Morphology.—In smears from serum bouillon or solid media, stained by an aniline dye or by Gam's method, one could recognise with difficulty a very small organism. Its size and shape could only be determined approximately under a magnification 1/12th objective and ocular No. 10, but as far as could be judged it was about 0·5 μ in size.

Toxin.—No toxin was demonstrable. Filtered cultures produced no disturbance when inoculated into goats in quantities of 8 c.c. subcutaneously, nor did filtrate produce any immunity.

VACCINATION OF GOATS.

Heated Cultures.—A seven days old culture in the seventh generation of seven days each was heated for half-an-hour at 56·0° F. Several goats were inoculated and some died of the typical condition.

The incubated tubes of heated culture gave a growth in 72 hours. Therefore half-an-hour at 56° F. did not destroy the organism.

A ninth generation culture seven days old was heated for one hour at 56° F. Control tubes remained sterile.

Sixteen goats received 1 c.c. each subcutaneously. There were a few slight temperature reactions only.

After two weeks four received 0·5 c.c. 11th generation culture subcutaneously. Three died, and the organism was isolated by

spleen cultures. The fourth lived after severe local and temperature reaction.

Three received a similar test three weeks after vaccination. Two died and the organism was isolated from the spleen. The third recovered after severe reaction.

Eight received a second dose of 2 c.c. heated culture as above, 14 days after first. There were no reactions, but three died of various other causes and the remaining five received a test dose of 0.5 c.c. culture four days after second vaccination. Two died of the typical disease and the organism was isolated from the spleen. Three recovered without reactions.

Thus, five goats remained to be hyperimmunised. These received 5 c.c. of culture, which they resisted, and later 10 c.c. Two died of other causes and three were placed in a herd of goats suffering from contagious pleuro-pneumonia. All succumbed to contagious pleuro-pneumonia in 15, 27, and 35 days respectively.

3. *Vaccination with Old Culture.*—Strain 142, second generation 37 days old, failed to give a growth when subinoculated into new media. Two goats each received 2 c.c. of this culture. One died of other causes and the other gave no reaction. On the 18th day it received 0.5 c.c. virulent culture and it succumbed on the eighth day with the typical condition, and pure cultures were isolated.

4. *Vaccination with Formalised Culture.*—Strain 142, 12th generation, was rendered avirulent by the addition of 0.1 per cent. formalin for seven days. Subcultures were sterile.

Two goats each received 8 c.c. subcutaneously. There was a slight temperature reaction in both cases. Both tested 14 days later with 0.5 c.c. culture of same strain 18th generation. One died on 13th day after temperature reaction and spleen culture was positive.

The other died of other causes.

Vaccination by Inoculation at Unsuitable Sites.—A characteristic of the result of subcutaneous inoculation of the organism is the rapidly spreading tense swelling. It was thought that, as in Bovine Pleuro-pneumonia, to which disease the present condition showed many similar characters, if a small quantity of virulent culture were inoculated in an area where there is little vascularity and the tissues are tense a slight immunity might have time to develop before the full force of the virulent culture was distributed around the body. Inoculations into other areas were also attempted. The results were interesting.

Goat 266 was scarified on the abdomen and 0.5 c.c. culture rubbed in.

Goat 279 was inoculated with 0.5 c.c. in the outer surface of the ear, against the cartilage.

Goat 260 was scarified on ear with 0.5 c.c.

Goat 269 was scarified at tip of tail with 0.5 c.c.

Goat 274 was inoculated at tip of tail with 0·5 c.c.

Goats 259 and 228, nostrils rubbed with 0·5 c.c. culture.

There were some slight thickenings and a little pain at each site but no rise in temperature. On the 14th day goats 260 and 274, and on the 15th day goats 266, 279 and 269, all showed a sudden rise in temperature between 104°-105·0° F. This was followed by several days (4-6) of high temperature, and then all became normal and remained healthy for two months. Some were tested with virulent culture while others were tested for their immunity to goat contagious pleuro-pneumonia.

Five were placed in an infected herd. Four died within three weeks of pleuro-pneumonia, and one resisted a month but was found to be a chronic case on slaughtering for examination.

Goats 259 and 228 gave reactions and were tested with 0·5 c.c. culture. They gave a slight temperature reaction and recovered. Neither of them succumbed to pleuro-pneumonia. It is possible that not sufficient time was given for immunity to develop if this organism was the causal agent of goat pleuro-pneumonia.

SUMMARY OF INOCULATIONS.

In all, 100 goats were inoculated with virulent material, *viz.*, organic emulsions, blood, culture, either subcutaneously, intrapulmonarily, intraperitoneally, or intravenously, and only eight resisted the infection and lived. Thus a 92 per cent. artificial transmission was obtained. Of those resisting infection five had received subcutaneous and three intrapulmonary inoculations.

Of 66 reactors inoculated subcutaneously as above, 26 showed a pneumonia and five an acute pneumonia with effusion of lymph. The remainder succumbed without lung lesions.

Of 22 reactors inoculated intrapulmonarily, 13 gave an acute fibrinous pneumonia and nine a slightly purulent pneumonia. Of two inoculated intraperitoneally and reacting, both showed slight pneumonia, and two inoculated intravenously gave similar results.

SUMMARY OF THE DISEASE.

An exceedingly virulent disease of goats has been under observation.

Sheep can also be infected artificially.

Cattle, pigs, guinea-pigs, and white rats are refractory.

Infection by contact failed.

Natural disease is not known so far.

The experimental disease is a septicæmia and pure cultures can be isolated from all organs.

The causal agent is a very minute cocco-bacillus.

The most favourable medium for growth is peptone bouillon with goat serum.

Vaccination against the organism has proved difficult, but small doses of virulent culture at sites unfavourable for rapid spread gave the best results.

DISCUSSION.

It will be realised by the reader that this artificial disease bears a very close resemblance to the artificial condition produced in cattle by the inoculation of virulent material or culture from cases of contagious bovine pleuro-pneumonia.

As Contagious Pleuro-pneumonia of goats closely simulates the bovine condition, is it not conceivable that the disease that has been studied in goats is the artificial condition of the Contagious Pleuro-pneumonia of that species and the organism described the cause of that disease?

In referring to the latter condition Mettam¹ states that "the disease is due to an ultra-microscopic and filterable virus as every attempt to cultivate the etiological agent has failed." On one occasion, however, he only transmitted the disease to healthy goats by intra-thoracic inoculation of filtrate, and it is not stated whether cultures of this filtrate were sterile.

As stated previously, a considerable time elapsed and many types of media were tried during the early part of this work before cultures were obtained from a strain of the artificial disease transmitted by inoculations of spleen pulp. Again, even with a successful medium, five and six days elapsed before opalescence was sufficient to enable a difference from control tubes to be detected, and probably many positive cultures were discarded too early.

Lanfranchi and Pacchiono in Italy, and Langhorn and Van Saceghan in the Belgian Congo, have isolated organisms said to be the cause of Pleuro-pneumonia, and therefore the consensus of opinion is not that "this disease must be classified as being due to an ultravisible virus" (Mettam). On the other hand, however, the writer has on many occasions failed to cultivate from natural cases of Goat Pleuro-pneumonia any organism simulating the one described above. But these attempts are being continued and there is some hope of success in the light of the new knowledge obtained.

The results of vaccination and hyperimmunisation with the organism in question in protecting goats against natural cases of pleuro-pneumonia were most disappointing, and though the writer was absent at the time he is prepared to accept that the deaths were due to this disease. A possible further theory is that Contagious Pleuro-pneumonia resembles Swine Fever and probably many other ultravisible diseases, in that a filterable virus and a *bacille de sortie* work in association with each other to produce the natural disease, and that the *bacille de sortie* in the case of Contagious Pleuro-pneumonia of goats is the organism under discussion.

¹Contagious Pleuro-pneumonia of Goats in East Africa. Paper No. 18, Pan-African Agriculture and Veterinary Conference, 1929.

OBSERVATIONS ON A PYOGENIC INFECTION OF SHEEP.

By WALTER JOWETT, F.R.C.V.S., D.V.H.

In a previously published description of certain pyogenic infections of sheep encountered amongst subjects passing through the Edinburgh Abattoir,* mention was made of a suppurative condition due to a Gram-negative bacillus; it is now proposed to give a short account of this disease and of its causal organism, the *Bacterium purifaciens* (Christiansen).

Several isolated cases of the infection now to be described have been observed from time to time, but nevertheless the disease would appear to be rather uncommon, at any rate amongst animals utilised for food purposes. So far the disease has been noticed as occurring only amongst the older sheep (ewes), and it is evident that under ordinary condition a lengthy period is required for the lesions to attain any considerable size. As a rule, the carcasses of infected sheep have been found in good condition, indeed some were said to be in excellent condition. It is possible, of course, that the disease might be much more serious in animals of low resistance or in those exposed to adverse influences.

Symptoms.—In most cases there was little or nothing to indicate the presence of the infection prior to slaughter, but if one had had opportunity to observe the subjects under natural conditions one might possibly have noticed hurried or difficult breathing since in the majority of cases the lungs were involved in the diseased process. In a few instances, however, it may be stated that one could have made a diagnosis during life, for in these there was noticed considerable thickening of the skin of the cheeks, together with the presence in the same region of numerous small abscesses—a pustular dermatitis in fact. In these cases swelling and abscess formation in the sub-mandibular region was also in evidence, as it was also in the throat, the subparotid glands being involved. In other subjects there was similar swelling of the parotid region but without abscess formation on the surface of the skin of the face. The visible symptoms in these cases were certainly sufficient to lead one to suspect the presence of the disease during life, provided, of course, that one had it in mind at the time of making the examination.

Lesions.—As previously mentioned, the commonest site was the lungs, together with, in the majority of cases, the related lymphatic glands. In every instance the outstanding feature which enabled the disease to be recognised at *post-mortem* examination was the peculiar physical property of the pus contained within the abscesses; so much was this the case that it became customary, as a matter of convenience, to refer to them as “plastic pus lesions.” The pus is pale green or greyish green in colour and

* *Journal of Comparative Pathology and Therapeutics*, Vol. XLIII, p. 109, June, 1930

of a plastic, dough-like, consistence, difficult to manipulate or spread on a glass slide when an attempt is made to prepare a smear for microscopical examination. The pus is quite inodorous, thus differing from that commonly observed in lesions due to the *Corynebacterium pyogenes*. As a matter of fact there is little likelihood of one confusing these two conditions, but in any event a microscopical examination would soon settle the point. The pus is different also from that present in the lesions of caseous lymphadenitis due to the Priesz-Nocard bacillus, in which disease the contents of the abscesses or lesions may vary from a greenish cream-like pus in recent lesions to a caseous or dry amorphous material, often stratified in those of longer standing. Moreover, the Gram-positive Priesz-Nocard bacillus can be detected in the pus or caseous material present in these lesions by microscopical and cultural examination. The lesions due to the *B. purifaciens* in the lungs occur usually in the form of multiple abscesses. In some cases these are discrete and of varying size from a pea to a hazel nut or even a hen's egg. There is usually some fibroid thickening around the abscesses, but otherwise the surrounding lung tissue often shows little change. In other cases, when the abscesses are multiple and situated close to each other, or where they have coalesced, there is frequently considerable alteration in the surrounding lung tissue, which may then be found to have undergone solidification and fibroid induration. In certain instances the bronchial and mediastinal glands were found merely enlarged, but in others these glands were markedly involved in the disease and on incision the peculiar plastic pus already referred to was found present in them. Similar abscesses to those encountered in the lungs have been observed in the kidneys, liver, and subcutaneous tissue of the head, flank, and elsewhere; also occasionally in the body lymph glands, notably the inguinal and pre-scapular.

Diagnosis.—To the remarks already made in this connection it may be added that cultural tests are necessary for an exact diagnosis, since microscopical examination of pus smears rarely gives satisfactory results. A few Gram-negative bacilli may be discovered amongst the degenerated pus cells but they are invariably very scanty and difficult of detection.

The Causal Organism.—From the pus in all the lesions examined a Gram-negative bacillus was isolated, usually in pure culture, and this was found to correspond more closely in cultural and bio-chemical characters with the *Bacterium purifaciens* (Christiansen) than with any other known organism; furthermore, like that organism it was found to be pyogenic on inoculation into sheep, and, as regards the smaller animals used for experimental purposes, was pathogenic only for mice. Difficulty was experienced in obtaining an authentic culture of the *Bacterium purifaciens* for the purpose of comparison, and, consequently, cultures

of the organisms isolated from Edinburgh sheep were forwarded to Dr. Magnusson, and at his suggestion also to Professor Christiansen, for an expression of opinion as to whether the organism in question corresponded with the one first described as the *Bacterium purifaciens* by the last-mentioned authority. As will be shown later, the respective organisms proved to be closely related or identical.

Morphology.—When examined in smears from pus the organism occurs as a short Gram-negative rod or as an ovoid bacterium. On agar and on Löffler's serum in primary culture it assumes a coccoid, an ovoid, or a rod-like bacillary form. Filamentous forms may also be seen in films made from cultures on these media (Fig. 1.). With some strains these filamentous forms at times were of considerable length; frequently they were sinuous, and occasionally seen stretching even up to half way across the field of the microscope. It was not unusual to find in the one film or cover glass preparation coccoid or ovoid forms, distinct rod-shaped bacilli, and long more or less sinuous filaments (Fig. 2). Frequently one observed the rod-shaped forms lying end to end to form a long chain, suggesting that they may have arisen from the disassociation of the filaments, or conversely, that the latter were formed by a number of the bacillary forms joined end to end. The occurrence of these various forms in the one preparation led at first to some confusion since it was erroneously assumed that they represented a mixed infection. Much time and trouble were expended in further plating in an endeavour to separate the different forms, but they persisted after repeatedly plating out, as they did also after passage through experimental animals (mice and sheep). One was therefore finally led to the conclusion that they represented merely different types or forms of the one organism, *i.e.*, a pleomorphic bacterium. In older cultures, especially those on serum-agar or Löffler's serum, the filaments at times assumed bizarre forms, resembling a long slender whip-lash, with perhaps one end swollen, like the handle of the whip. These were taken as representing involution forms.

In films from bouillon cultures the organisms appeared in bacillary form, and it was not unusual to find them lying end to end in long chains of ten to twenty segments or even more.

Cultivation.—The organism is an ærobe and a facultative anærobe. Its optimum temperature is about 37° C. Little or no growth occurs at room temperature. It can be cultivated without special difficulty on the ordinary laboratory media, growing well on solidified serum, agar, serum-agar, and in bouillon. On agar plates after 24 hours incubation at 37° C. rounded greyish-white colonies are seen. They have no very special characteristics but are sharply outlined, and measure at this stage up to 1 m.m. in diameter, and after 48 to 72 hours incubation may attain the size of 2 m.m. or rather more. The colonies are moist in appearance

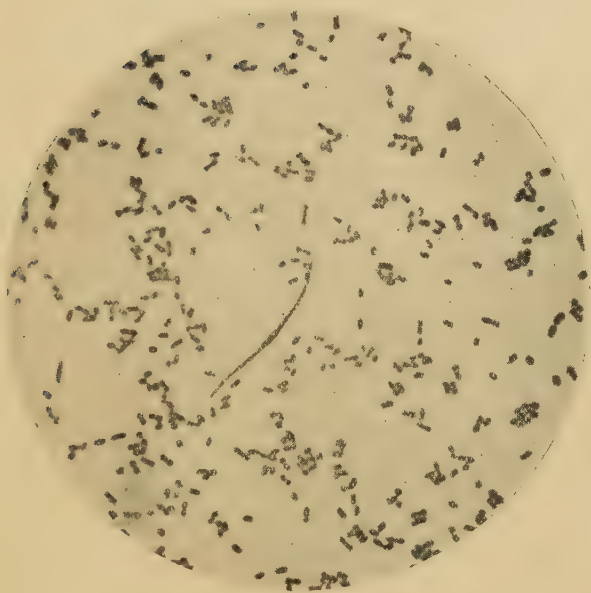


FIG. 1. $\times 1300$.

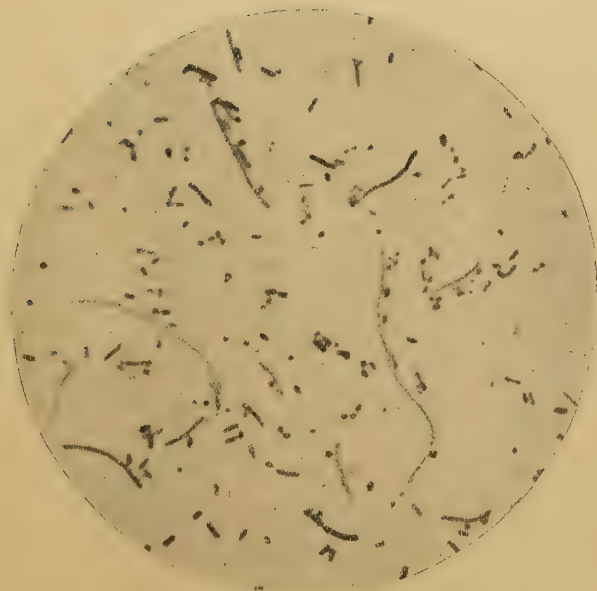


FIG. 2. $\times 1300$.

and can be readily fished off with the platinum needle. When subcultured on to agar slants the growth assumes a greyish-white appearance, in the form of a film which does not spread very far beyond the area of insemination. When examined with a hand lens it is not uncommon to find that the film is made up of innumerable minute colonies which tend to retain their individuality, at any rate during the earlier stages of incubation. Later, of course, they may coalesce to some extent to form a film, but this is never very profuse, and it is frequently possible to distinguish many individual colonies at the edge of the film even after some days incubation. The water of condensation is faintly cloudy, with usually some amount of sediment in its depth. On serum-agar growth is rather better than on plain agar but is otherwise similar. In stab agar it is nail-like. On blood-agar the organism grows readily in the form of a layer of ivory or white colonies which generally tend to retain their individuality. There is no hæmolysis and no colour change. On Löffler's serum the organism grows quite well in the form of greyish-white colonies of moist appearance which may coalesce to form a film. There is no "pitting," *i.e.*, no evidence of proteolytic action. In bouillon a distinct sediment is formed, the medium remaining hazy. There is no distinct surface pellicle. On gelatin at room temperature there is little or no growth. Litmus milk does not appear to be very favourable to growth and usually this medium remains unchanged. If the incubation be prolonged for several days acid formation may be evident in the case of the more vigorously growing strains.

Fermentation Reactions.—All the strains tested readily fermented glucose, maltose, mannite, and saccharose, producing acidity in 24 hours at 37° C. The effect on lactose was tardy and acid was evident only after some six days incubation at 37° C. Dulcitate, adonite, and salicin were not fermented. None of the strains produced gas.

Professor Christiansen, to whom two strains were forwarded for examination, obtained similar results, and in a communication in this connection states that he found "complete correspondence" (*i.e.*, with his strain of *B. purifaciens*), "including the characteristic slow fermentation of lactose, and the effect of the cultures on the three pentoses arabinose, xylose and rhamnose (iso-dulcitate), of which only xylose is fermented, the other two substances not being fermented." He concluded that the strains in question were identical with the *B. purifaciens*. "They are," he remarks, "perhaps more inclined to form chains or filaments in bouillon" (*i.e.*, than was the organism he originally described), "but," he continues, "the *B. purifaciens* is also a rather pleomorphic bacterium."

Pathogenicity.—The virulence of the different strains isolated was tested on guinea-pigs, rabbits, and mice. Inoculation into guinea-pigs, either subcutaneous or intraperitoneal, proved without

effect, whether pus or cultures of the organism were used. Rabbits likewise proved insusceptible to intravenous, subcutaneous, or intraperitoneal inoculation.

In mice the subcutaneous inoculation of cultures gave either negative results or produced merely dullness ending in recovery, but the intraperitoneal injection of relatively large doses of culture killed these animals in 24 to 36 hours, the organism being recoverable from the heart's blood.

Sheep were inoculated with the following results :—

May 22nd, 1930. Sheep No. 1.—Inoculated intravenously (and also partly subcutaneous) with a suspension in saline of a 24 hours growth on agar of the organism isolated from a naturally infected sheep. Killed June 3rd. Two abscesses at site of the subcutaneous inoculation. Each contained plastic viscous pus from which the *B. purifaciens* was recovered in pure culture.

June 12th, 1930. Sheep No. 2.—Inoculated with suspension in saline of growth from 24 hours agar culture, 1 c.c. intraperitoneally and about the same subcutaneously. Killed August 26th, 1930. No evidence of abscess formation.

This strain was evidently of low virulence. It had been maintained in the laboratory for some months and may have become attenuated in consequence.

June 12th, 1930. Sheep No. 3. Inoculated, with 1 c.c. of an 18 hours old serum bouillon culture (a recently isolated strain) subcutaneously right submaxillary region, and at same time scarifications were made on inner surface of lips and some of the culture applied to the wounds by means of a swab. Killed August 26th. Abscess in right submaxillary region, otherwise normal. The abscess contained the usual plastic pus, from which *B. purifaciens* was recovered in pure culture.

Agglutination Tests.—An agglutinating serum was obtained by inoculating a rabbit with the surface growth of agar cultures of the organism isolated from one of the Edinburgh sheep. Living cultures were used for this purpose from the commencement. The first injection was given intravenously, this being followed by intraperitoneal and subcutaneous inoculations at weekly intervals, the course lasting from five to six weeks. The serum then agglutinated the homologous strain at 1 : 960 and the other (Edinburgh) strains tested at somewhat lower dilutions. The different strains were therefore accepted as being identical. A Swedish strain obtained through the courtesy of Dr. Magnusson was also agglutinated but in lower titre (up to 1 : 120). Some difficulty was experienced in all these tests by reason of the occurrence of spontaneous agglutination, but they tended nevertheless to indicate the identity of the different strains tested.

Professor Christiansen carried out agglutination tests on two strains forwarded from Edinburgh, utilising for the tests serum from a rabbit immunised by inoculation with cultures of a Swedish

strain of *B. purifaciens*, and in a communication to the writer in this connection states that the serum so obtained agglutinated the homologous strain completely at 1 : 1,000, whilst the effect on the strains received from Edinburgh was weaker—1 : 100 in one and even less in the other. In concluding his remarks with regard to the identity of the respective organisms Professor Christiansen observes "It thus seems as if there are various serological types of the *B. purifaciens*, a fact which in itself seems very reasonable."

Dr. Magnusson also kindly carried out agglutination tests at Malmö on three strains received from Edinburgh, as well as on a strain he himself had isolated from a sheep in Sweden, and in respect of these experiments states "I have made agglutination tests with serum from two sheep. One of the sheep was inoculated with my own new strain and the other with the Edinburgh strain No. 30. Serum from both sheep gave reactions for the three Edinburgh strains as well as for the Swedish strains. I am therefore sure they are all identical."

The identity of the respective strains with the *B. purifaciens* (Christiansen) is thus clearly established.

Viability.—The organism is a delicate one and is easily killed by adverse influences. It can be maintained in culture in the incubator at 37° C. for a fairly long period if transplanted to fresh medium at intervals of a week or so, but after that time gradually dies out. At room temperature cultures soon lose their vitality.

It seems likely that the disease originates in the first place as a wound infection. From the primary focus, however originated, there is no doubt that metastasis is effected by the lymphatic route. The disease, as already pointed out, is of slow progress, at any rate under ordinary conditions, and apparently it does not occur as a bacteraemia.

In conclusion, the writer desires to acknowledge his indebtedness to Mr. E. J. H. Sewell, M.R.C.V.S., for notes taken at the post-mortem examination of the naturally infected sheep. As previously mentioned, Dr. Hilding Magnusson and Professor Christiansen kindly examined and verified the identity of some of the cultures of the organism isolated, and to both these authorities the writer is indebted.

The investigations were conducted in the Laboratory of the City of Edinburgh Veterinary Department.

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PARAGONIMUS WESTERMANNI IN A CAPTIVE TIGRESS.

By C. A. MCGAUGHEY.

*From the Department of Bacteriology and Preventive Medicine,
University of Manchester.*

THE occurrence of this parasite in Great Britain has already been recorded by Vevers (1923), who found it in captive wild animals at the Zoological Gardens, London. As it seems to be comparatively rare in this country it was thought that a short description of a case of paragonimiasis in a captive tigress might be of interest.

The subject was an adult tigress purchased by Belle Vue Zoological Gardens, Manchester, in 1925. The diet had consisted entirely of horse flesh. About June, 1928, the attendant noticed that she had a heavy, greenish-yellow, mucous, nasal discharge and that her breathing was laboured. After a week or two of illness she seemed to recover, but two or three months later became ill again, showing similar symptoms. Death occurred on December 17th, 1928.

Post-mortem examination was made by Mr. Craig, M.R.C.V.S., who found the submaxillary glands enlarged and fibrosed, and the lungs consolidated, dark brown, tough, and leathery.

A piece of lung about eight inches long, eight inches broad, and two inches thick was sent to the laboratory for further examination. Several small cysts, each about the size of a broad bean, were noticed bulging the pleural surface of the lung. On cutting into these cysts two parasites were found in each cavity (Fig. 1)

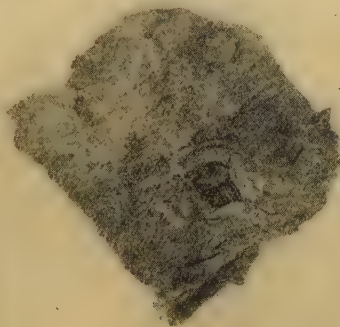


FIG. 1.
Opened cyst with two parasites (half actual size).

and also a small quantity of reddish-brown fluid which contained numerous eggs. About 18 parasites altogether were found, all apparently mature and of recent development. In the depths of the lung tissue several smaller cysts containing calcified dead parasites were found. Several pieces of lung were fixed in ten

per cent. formol saline, embedded in paraffin, and examined microscopically.

Histological examination showed that the cysts were dilatations of bronchioles (Fig. 2). The walls of the bronchioles were fibrosed, the muscular coat had disappeared, and there was infiltration of the wall with lymphocyte and plasma cells. In some places the mucosa was raised into papillary projections and anaplasia of the

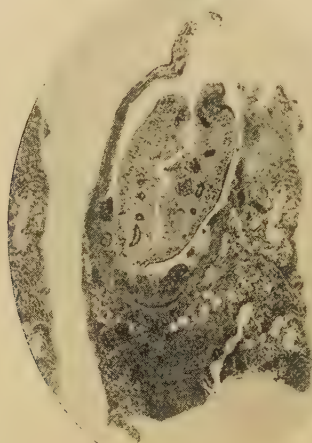


FIG. 2.
Cyst and parasite divided longitudinally.

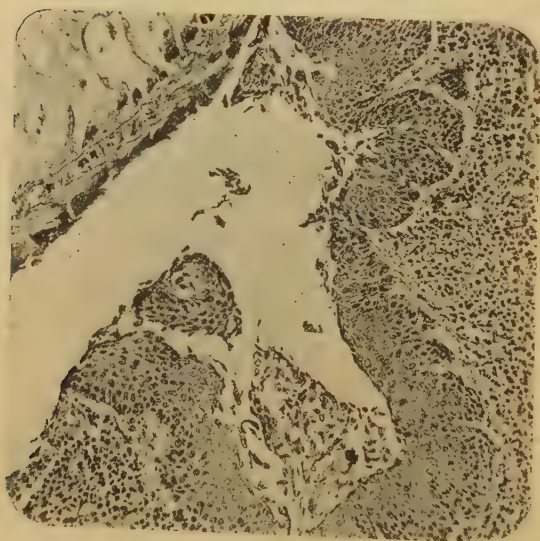


FIG. 3.
Shows anaplasia of the lining membrane of the cyst and the scale-like spines of the parasite ($\times 120$).

lining membrane was a striking feature. Fig. 3 shows the squamous epithelium lining a cyst. Examination of the alveolar tissue revealed an organised pneumonia, the alveolar walls being thickened, fibrosed, and infiltrated with cells.

The parasites conformed to the description of *Paragonimus westermanni* given by Brumpt (1922). They were about the size and shape of coffee beans, *i.e.*, about 10 mm. long, 6 mm. broad, and 3 mm. in thickness, reddish-brown, almost transparent when fresh, opaque when fixed in alcohol. Microscopical examination showed the anterior and ventral suckers, the branched intestine, and the scale-like spines on the outer tegument (Fig. 3). The eggs were ovoid, about 100μ long, 65μ broad, and light brown. Many showed opercula (Fig 4).

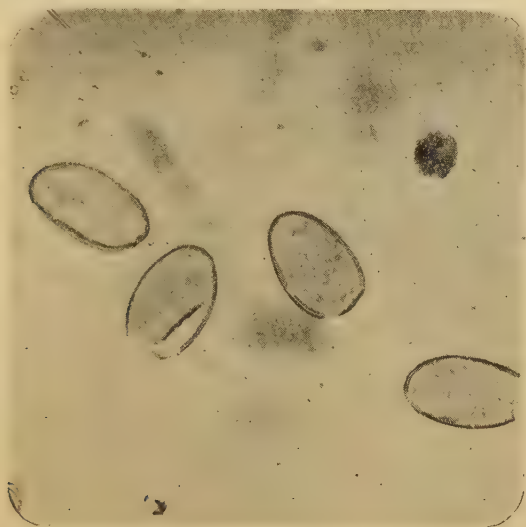


FIG. 4.
Ova of *Paragonimus Westermanni* ($\times 180$).

Discussion.—This parasite is one of the Platyhelminths of the order Trematoda, genus *Paragonimus*. Three different species have been recognised by Ward and Hirsch (1915):—(1) *P. westermanni* found by Kerbert in 1878 in a Bengal tiger; (2) *P. ringeri* found by Cobbold in 1880 in man, and (3) *P. kellicotti* found by Ward in 1908 in U.S.A. in the pig, dog, and cat.

Ward and Hirsch differentiated the three species by the arrangement of the spines and by certain features of the alimentary tract. Vevers (1923) supported this view, also finding differences in the form of the eggs. He also distinguished a fourth species, *P. compactus*, found in the Indian mongoose. According to Kobayashi (1919), the specific characters described by Ward and Hirsch are only simple individual variations. In Korea this

observer found the same species in man, pig, dog, and cat.

Life History.—On account of the great prevalence of paragonomiasis in the natives of the Far East, a great deal of research on the evolution of this parasite has been carried out by numerous Japanese workers, but the complete life history has not yet been fully worked out. The main facts discovered, as given by Brumpt (1922) and Nakagawa (1917), are as follows.

The *miracidium* emerges from the egg after 16 days in summer or 60 days in winter, and seeks out its first intermediate host, which may be any one of six or seven species of mollusc. It enters the mollusc and forms under its skin a *sporocyst* which generally gives rise to a single *redia*. The *redia* migrates in the tissues of the mollusc and by internal budding produces *daughter rediæ*, which lodge in the liver-pancreas and give rise to *cercaria*. The cercaria leave the mollusc and enter the second host, which may be any one of several species of crayfish or crab. They encyst in the muscles of the trunk and after 42 to 54 days become infective. If the infected crustaceans are ingested by cats or dogs the *metacercaria* are set free in the small intestine, perforate the alimentary tract, get into the abdominal cavity and the thoracic cavity, where they lodge for about 30 days; they then travel through the pleural surface of the lungs and become fixed in pairs in the bronchioles, which they hypertrophy and transform into cysts. It is thought that the parasites reach the lungs in human beings in a similar manner. Localisation in other organs appears to be accidental.

The disease in man caused by this parasite is called pulmonary distomatosis or parasitic hæmoptysis. It occurs in China, Korea, Formosa, Japan, Indo-China, Phillipine Islands, Peru, Yucatan, and Mexico. Human beings become infected by eating badly cooked or raw crabs or crayfish.

Symptoms.—Chronic cough and expectoration of reddish-brown sputum. Hæmoptysis produced by rupture of small blood vessels may last four or five days. The disease in some cases resembles tuberculosis but the patients emaciate much more slowly. Cerebral distomatosis is a complication noted by several authors and usually gives rise to symptoms of epilepsy. In these cases cysts containing parasites and their eggs, or the latter only, are found in the brain.

Diagnosis is made by examination of the sputum for eggs, which should be carefully measured and should show opercula.

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ARE HENS SUSCEPTIBLE TO INFECTION WITH THE BACILLUS OF JOHNE'S DISEASE (PARA-TUBERCULOSIS) ?

By Dr. T. VAN HEELSBERGEN.

(*From the Institute of Parasitical and Infectious Diseases of the Utrecht University. Dir. Prof. Dr. L. de Blicck.*)

A PARTICULARITY which has attracted the attention of several investigators is that cattle affected with para-tuberculosis generally give a good reaction to injection with avian tuberculin.

Bang was the first who paid attention to this, and the fact has been confirmed several times. Especially American investigators were bent upon comparing the value of avian tuberculin and Johnin (para-tuberculin) with reference to para-tuberculosis in cattle.

As far as one may rely on the obtained results, the value of the two is almost identical, which means that cattle suffering from para-tuberculosis give an equally "pronounced" reaction to an injection with avian tuberculin and to a tuberculin prepared from para-tubercle bacilli. This indicates a close bio-chemical relation between the two micro-organisms. In this respect they are probably more closely related than, *e.g.*, the bovine and the avian tubercle bacilli. In general, cattle affected with bovine tuberculosis give a "slight" reaction to the avian tuberculin, whilst hens affected with avian tuberculosis do not react nearly so well to the bovine tuberculin as to that of avian origin.

Besides this biochemical relationship of the two micro-organisms in question, the diseases caused by them are also similar in some respects.

These two micro-organisms are able to give rise to a leprosy-like affection: slight inflammatory symptoms with an equal richness in bacilli. It is true that these forms in avian tuberculosis are not the rule, but they occur, and are frequently observed in ducks. It is therefore not surprising that many investigators gave it as their opinion that the two microbes are varieties of one and the same organism. Nowadays, however, this view is almost abandoned.

Morphologically, there is a great difference between the two bacilli. The avian tubercle bacilli show themselves in the bird as rather long, slender rods, but one sometimes finds rather thick forms. Very often granulation appears. The granules show themselves in rows, the bacillus resembling a streptococcus, or they appear independently. In these cases the granules may show a smooth rim or wing-like faintly-coloured appendices on both sides. The granules may also appear like spores situated at one pole. The para-tubercle bacilli do not often show this beaded appearance. On the other hand, they show a stronger tendency to form small clumps, whilst the bacilli are in general much thinner and shorter than the avian tubercle bacilli.

Bacilli morphologically identical with the para-tubercle bacilli are found in the slimy membrane which is generally to be seen

at the opening of water-taps when these are used rarely ; so as far as I know, however, these are pure saprophytes, but the resemblance to para-tubercle bacilli is so striking that a closer study of these acid-fast water bacilli would be very interesting.

Pathogenicity.

As well as in cattle and sheep, the para-tubercle bacillus is capable of stimulating typical symptoms after artificial infection in goats, rabbits, mice, and rats. As far as I know, no attempts have been made to infect hens with the cause of Johne's disease.

The pathogenicity of the avian tubercle bacillus covers a larger field. Besides all kinds of birds, hogs, cattle, horses, monkeys, and men may be affected spontaneously with this bacillus. Rabbits, mice, and rats are susceptible to experimental infection. Guinea-pigs offer a stronger resistance to the infection by avian tubercle bacilli, which does not mean, however, that they are immune to it.

Dogs and cats are insusceptible.

In general, hens are not so susceptible to tubercle bacilli of the mammalian type. It was supposed for a considerable time that they were immune to this type of tubercle bacilli. Later on, investigators stated, however, that after a large dose emaciation appears, and in several organs the bacilli are present in large numbers. At any rate, one may admit a very strong resistance of the fowl against the mammalian tubercle bacillus.

The following experiments were made in order to obtain an answer to the above question :—

From February 18th to 25th, 1930, 13 full grown hens were fed with crushed intestine from bovines suffering from para-tuberculosis. The intestine contained a great number of para-tubercle bacilli, and a large quantity of culture was added to it. Three other hens were inoculated subcutaneously with para-tubercle bacilli, and one hen was inoculated intravenously. In the latter experiments the para-tubercle bacilli came from intestinal parts treated with antiiformin (20 per cent.) solution for ten to fifteen minutes.

At the end of September, seven months after the infection, all the hens were killed. When alive these birds had never shown any symptom of infection. The egg-laying had been regular and the general condition had been excellent.

At the post-mortem examination of these 17 hens not a single trace of disease was met with. Some of the hens showed a not severe enteritis in consequence of coccidiosis or nematodes. Inflammatory zones in the organs did not exist. The bone-marrow in all hens was normal.

Microscopical preparations were made from different parts of the intestine, from all organs, and from the bone-marrow, but without discovering the slightest trace of para-tubercle bacilli. In the intestine, however, acid-fast bacilli were occasionally noticed in small numbers, but these microbes were too large to pass for para-tubercle bacilli.

These experiments proved that full-grown hens are insusceptible to infection with para-tubercle bacilli. The para-tubercle bacilli which had been introduced evidently disappeared from the body without leaving a trace.

Is ingestion a reliable means of infecting hens with tuberculosis or with para-tuberculosis?

This question is put here because in the above experiments the greater part of the bacilli were given by the mouth.

Early in 1928, 15 full-grown hens had also repeatedly been given virulent avian tubercle bacilli *per os*. The infection took place as follows:—

To their food was added a crushed tuberculous liver of a hen, and this was repeated three times. About eight months after the infection the hens were killed and the intestine, organs, and bone-marrow were microscopically examined in order to discover the possible presence of tuberculous alterations or tubercle bacilli. The result of this examination was that in three of the fifteen hens (20 per cent. of the cases) neither tuberculous lesions nor tubercle bacilli were found. However, small tuberculous lesions may have been overlooked. Besides, experiments have not been numerous enough to draw definite conclusions.

It is also possible, that with a longer period of observation there would have been more positive results. These suppositions cannot be taken for certain, however. After feeding full-grown hens with tubercle bacilli it will be wise as yet not to expect a 100 per cent. result.

Possibly young fowls may be more susceptible and, from this point of view, it may be necessary to repeat the experiments with para-tubercle bacilli on chickens.

REVIEW.

Lehrbuch der Vergleichenden Mikroskopischen Anatomie der Haussäugetiere. Bearbeitet von Dr. med. A. Trautmann, Professor an der Tierärztlichen Hochschule in Hannover, und Dr. med. u. dipl. Tzt. Jos. Fiebiger, Professor an der Tierärztlichen Hochschule in Wien. Berlin, 1931. Verlag Paul Parey. 26 Marks.

With a slight change in title, this work represents the 6th edition of Ellenberger's "Grundriss der Vergleichenden Histologie der Haussäugetiere," which was first published in 1888, and in successive editions has maintained the first place among textbooks on the same subject in any language. It retains the general form of the immediately preceding edition, of which also Professor Trautmann was co-editor. The text extends to 405 large 8vo pages, and the illustrations, some of which are coloured, now number 447. A number of these are new, and all command admiration for their clearness and accuracy. The work reflects great credit on both editors and publishers.

ABSTRACTS.

COWS INFECTED WITH STREPTOCOCCUS EPIDEMICUS.

By W. D. FROST, R. C. THOMAS, MILDRED GUMM, and F. B. HADLEY.

IN this paper the authors record the occurrence of the *Streptococcus epidemicus* Davis in the milk of 17 cows. Two of the cases were associated with an epidemic of septic sore throat. The other 15 cows were giving milk which, mixed with a considerable quantity of other milk, was used as food. No human infection was produced so far as could be determined. Eight of these cows were found, during the course of four years, in certified herds of approximately 1,300 cows which had been regularly tested at monthly intervals. The other seven were accidentally discovered in small herds.

The authors' observations would indicate that cows infected with *Streptococcus epidemicus* are rather widely distributed, and that this streptococcus undoubtedly occurs quite generally and frequently in milk used for direct consumption, as well as in that which is used for cheese and butter making, without producing disease. The infection of cattle with this human type of streptococcus apparently begins as a mild disease, which may apparently continue a mild course or become very severe. There is a tendency for the infected portions of the udder to lose their milk-secreting function. In the mild cases, to which most of the authors' cases belonged, there is little or no change from the normal in either the udder or the milk, although *Streptococcus epidemicus* may be excreted in large numbers.

Of the 13 cows in which the extent of the infection was determined, seven cows, or 53 per cent., were infected in only one quarter, four cows, or 31 per cent., were infected in two quarters, and two cows, or 16 per cent., were infected in three quarters, although the infection in the different quarters did not always occur simultaneously.

The strains of *Streptococcus epidemicus* are apparently all virulent and are identical with those strains isolated from epidemics in either man or cow. (*Journal of Infectious Diseases*, Vol. 46, 1930, pp. 240-252.)

INVESTIGATIONS REGARDING SWINE FEVER.

By W. DAVID and M. SCHWARZ.

THE authors have made extensive experimental researches regarding swine fever determined by inoculation of the virus contained in a filtrate, and the results are summed up as follows.

The observations and experiments were made on 286 animals infected with bacteria-free filtrates.

The onset of the infection after subcutaneous inoculation is already shown on the second or third day by conjunctivitis, and from the third to the fifth day by fever (over 40° C.) before any other signs are observable.

Very early, even after infection with bacteria-free virus, there occurs a secondary polybacterial infection in which organisms of the paratyphoid and bipolar kind play a special rôle.

From about the seventh to the tenth day after infection this secondary infection occurs in the majority of virus-infected animals, which fact shows the necessity of early intervention with immune serum and also explains the failure of serum treatment in single animals or entire herds.

In virus-infected animals without secondary infections (the pure virus disease) changes in the lymphatic glands are among the earliest and most striking.

The earliest changes are at first not characteristic in all organs. The characteristic (septicæmic) symptoms are often unspecific—swelling and hyperæmia of the lymphatic glands and catarrh of the mucous membrane of the digestive system.

The earliest characteristic changes appear at different times according to the virulence of the virus and the susceptibility of different animals. In some animals they may be shown in different organs as early as the fourth day, but as a rule during the first few days they are so sparingly present that the most searching examination is necessary to ensure that they are not overlooked.

These early characteristic changes do not appear regularly at definite places, but certain organs and groups of lymphatic glands appear to be preferred sites. (The lymphatic glands in the neck and at the entrance to the chest, also the glands of the kidneys and rectum; the spleen, kidneys, urinary bladder, stomach, large intestine, and skin.)

Hæmorrhagic and anæmic infarcts in the spleen are of great value for diagnosis, but they occur less frequently after natural infection than after artificial infection. In the latter they are almost constantly present.

Regressive changes in the mucous membrane of the digestive apparatus and, above all, diphtheroid centres occur both in the pure virus disease and also in consequence of the secondary bacterial infection. These lesions are most frequent in secondary infections with different paratyphoid bacteria, and secondary infections may occur to an enzootic extent in swine fever outbreaks.

Hæmorrhagic-croupous pneumonia occurs both in the pure virus disease and in cases with a secondary bacterial infection. A croupous pleuro-pneumonia with a tendency to necrosis, caused by a bipolar infection, may prevail to an enzootic extent in swine fever, and in certain circumstances be mistaken for so-called swine plague (*Schweineseuche*).

As a result of their observations regarding swine fever pneumonia the authors think that in every case in which the history and the lesions appear to point to acute swine plague it is absolutely necessary to suspect swine fever and to carry out the proper search for the virus of that disease, using all the known means that can be employed for the purpose. If that is not done there is a danger that the existence of swine fever may be overlooked, with consequent spread of the disease. (*Archiv. für wissenschaft. u. prakt. Tierheilk.*, Vol. 62, 1930, pp. 315-343.)

COMPARATIVE VALUES OF ANTI-ANTHRAX SERUM AND OF NEOSALVARSAN IN THE TREATMENT OF EXPERIMENTAL ANTHRAX.

By TIMOTHY J. KUROTCHKIN and HOBART A. REIMANN

THE authors observe that a case of cutaneous anthrax in the hospital in Pekin was apparently successfully treated with neosalvarsan, and since an account of it was published two other cases have been observed. One, an advanced case with a pustule on the neck, died in spite of intensive treatment with both immune serum and neosalvarsan. The other, an early case with a pustule on the eyelid, recovered after receiving ·6, ·9, and ·9 gm. of neosalvarsan on three successive days. An experimental study was then

made to determine the comparative value of anti-anthrax serum and of neosalvarsan in treating anthrax in mice, guinea-pigs, and rabbits.

Methods.—The strain of anthrax bacilli used in this study was obtained from the pustule of a fatal case of anthrax. Mice died within two days after the subcutaneous inoculation of ·000001 c.c. of a 24 hour agar slant growth suspended in 5 c.c. physiologic salt solution. Guinea-pigs succumbed within three days after inoculation with ·000005 c.c. Two kinds of commercial anti-anthrax serum made by well-known companies and the neosalvarsan of Billon were used in the tests.

As a rule, the virus was inoculated subcutaneously so that ·2 c.c. of physiologic salt solution contained the desired amount of bacilli for mice, and 0·5 c.c. the amount for guinea-pigs and rabbits. Anti-anthrax serum and neosalvarsan were injected intravenously into the tail veins of mice, heel veins of guinea-pigs, and the ear veins of rabbits. The neosalvarsan was dissolved in sterile physiologic salt solution immediately before use.

Experiments with Mice.—It is well-known that anti-anthrax serum is of no value in protecting mice from anthrax infection since they are too highly susceptible. In spite of this, however, comparative experiments were made to determine if either anti-anthrax serum or neosalvarsan had any therapeutic effect whatever in prolonging life.

Eighty-five white mice, averaging 15 grams in weight, were infected with varying amounts of bacilli (from 1 : 500,000 to 1 : 1,000,000 c.c.). Serum was administered intravenously in varying amounts corresponding to from 1 c.c. to 13 c.c. per kilo of weight. In several instances serum was given immediately after inoculation with bacilli but usually from half-hour to three days before infection. Without exception all animals died within 24 to 36 hours from anthrax septicæmia.

Mice were then given ·000005 c.c. of anthrax bacilli subcutaneously and were immediately injected with varying doses of anti-anthrax serum or of neosalvarsan, and the results showed that these were of no therapeutic value in mice infected in that way.

Experiments with Guinea-pigs.—In similar experiments with guinea-pigs the whole of the animals (26) treated with serum died within 72 hours. On the other hand, the experiments indicated that neosalvarsan was of value in the treatment of guinea-pigs. The drug was injected intravenously immediately after inoculation of the bacilli and 24 animals were used in the experiment, the dose of the drug varying from 2·5 mg. per kilo to 20 mg. The three animals that received the smallest dose died in three days, but of the 21 which had been given the largest doses twelve survived. Nine died in three or four days. All the untreated controls died. It was, therefore, clear that in guinea-pigs neosalvarsan is of value in the treatment of anthrax infection, since many of the animals survived after one injection of the drug although comparatively large quantities of virulent bacilli were given.

Experiments with Rabbits.—Since rabbits are even more resistant than guinea-pigs to artificial anthrax infection, it was expected that the therapeutic administration of neosalvarsan would be still more strikingly beneficial and that immune serum would also be of some value. Schuster has already reported the successful treatment of rabbits with salvarsan. Twelve rabbits were given doses of anthrax bacilli similar to those given to guinea-pigs and immediately afterwards injected with immune serum. From 1 to 10 c.c. of serum per kilo was given. All of the animals died within five days from anthrax septicæmia.

Sixteen rabbits received the same amount of bacilli as in the preceding

experiment and were then injected with amounts of neosalvarsan ranging from 2.5 mg. to 20 mg. per kilo. Within eight days all of the animals succumbed to the infection.

Thus neither immune serum nor neosalvarsan had any effect on the course of anthrax infection in rabbits, although death was delayed in a few of the animals that received the drug.

The authors conclude that, in spite of the negative effects of the specific immune serums which they used, it cannot be assumed that serum is useless in the treatment of other species, since it has been found to be of value in other domestic animals and numerous statistics appear to indicate its usefulness in treating human anthrax. They believe that it is difficult to predict the value of any measures in the treatment of human anthrax infections from the results and experiments on other animals, as each species apparently reacts in a different manner to therapeutic efforts. (*Jl. of Infectious Diseases*, Vol. 46, 1930, pp. 36-41.)

POSSIBLE SPREAD OF FOOT-AND-MOUTH DISEASE THROUGH COW-POX VACCINE.—RECENT EXPERIENCES IN NORWAY.

By H. MAGNUSSON.

MAGNUSSON (Malmö, Sweden), commissioned by the Norwegian Government, confirmed the diagnosis of the veterinary officials of that Government in regard to the appearance of foot-and-mouth disease among several cattle inoculated at the Oslo Veterinary Clinic with cow-pox vaccine obtained from the Public Health Institute of Norway. The diagnosis was also confirmed afterwards by Schmidt-Jensen (Denmark).

In Scandinavia the disease has seldom occurred. Until 1926 and 1927 it was unknown in Norway; during this period 117 cases were detected and the disease was quickly eradicated by slaughter. In the southernmost part of Sweden there occurred successively, in 1925 and 1926, two extensive outbreaks, the first caused by type O and the second by type A virus. Between June, 1927, and December, 1929, only four cases were detected. The epizootic prevailing at present in Northern Germany has been responsible for four isolated cases between December, 1929, and April, 1930. The disease was stamped out on these eight occasions by slaughter. The virus was of type O.

The occurrence of the disease in Norway was not connected in any way with the presence of the disease in Sweden, but was proved beyond doubt to be derived from the vaccine lymph which has been used for many years to vaccinate children and which has been propagated for a long time on calves. Four different samples of the vaccine were tested and all were found capable of setting up typical foot-and-mouth disease in cattle. One of these samples had been preserved in glycerine at low temperature for 18 months; nevertheless, it set up in three animals, by intravenous inoculation or scarification, generalised foot-and-mouth disease within three or four days.

The discovery was made accidentally in undertaking experiments upon cows to ascertain whether they could be vaccinated preventively against outbreaks of cow-pox with udder inflammation. The calves or heifers used for the production of the vaccine were subjected to the customary veterinary inspection (before inoculation and after harvesting the vaccine) were slaughtered within 24 hours after the taking of the vaccine. Moreover, in the course of the year the cows showed no symptom whatever of foot-and-mouth disease. The discovery of Belin's so-called *complexe vaccino-aphteux* could therefore not be made with absolute certainty

by the veterinary inspection. In such cases a control test, by scarification of the buccal mucous membrane or intravenous injection of the vaccine pulp, would be advisable.

Magnusson tested the Norwegian virus upon guinea-pigs in his laboratory. After scarification of the pads it produced typical local lesions and, afterwards, a generalised infection in two to three days in 100 per cent. of the inoculated animals. In the tests carried out in Norway vaccine that had been preserved for 18 months produced large vesicles on the tongue of inoculated cattle. With an extract obtained from epithelial shreds taken from the vesicles Magnusson inoculated five guinea-pigs; at the first passage local lesions appeared in all five and generalised infection in four. All died of cachexia, from the tenth to the fourteenth day.

Further experiments were carried out to exclude the possibilities of equine vesicular stomatitis and the generalised exanthema of cow-pox by means of immunity tests with the different types of virus hitherto known. Nineteen guinea-pigs recovered from type O (generalised infection) proved immune towards infection with the Norwegian virus. Seven recovered from type A (generalised infection) proved susceptible (with generalisation) to the virus (except one, which died prematurely). Likewise, four recovered from type C infection proved susceptible. In the case of guinea-pigs recovered from infection with the Norwegian virus three proved resistant to type O infection, while eight others proved susceptible to type A and three to type C. The Norwegian virus was, therefore, of pure O type.

Thus, this Norwegian lymph, which contained the foot-and-mouth virus in abundance, had been inoculated into many thousands of Norwegian children, but, so far as is known, never set up foot-and-mouth disease. Magnusson claims that this observation lends further support to the view which he has expressed, that, from the practical standpoint, the disease is not transmissible to man. The case found at Rheims, described quite recently by Trautwein, is hitherto the only published one, he believes, which can be deduced as proof of the possible transmission of the infection to man. Transmission of the disease by ingestion of infected milk or by contact, without concurrent infection through a wound, seems to him to be improbable. The numerous descriptions of this kind found in the medical and veterinary literature are of doubtful authenticity and have not been subjected to sufficient control. In the same literature there are described, however, in fact, several analogous examples of contamination of vaccine lymph with the foot-and-mouth virus. In 1898 circumstances of this kind occurred in Switzerland, in 1908 in the United States of America, and in 1917 in Hungary.

Thanks to the interesting researches of Loeffler and Frosch, in 1897, and, especially of Belin, in France, and later, of Ducloux, Rinjard and Cordier, in 1929, it has been found that the foot-and-mouth virus thrives remarkably well in symbiosis both with the cow-pox virus and with the sheep-pox virus. However, the rules that have been drawn up for the preparation of cow-pox lymph have not anticipated the possibility that a similar symbiosis may be brought about spontaneously and against all expectation. A well-known expert in such questions, Professor Gins, of Berlin, has difficulty in believing that a symbiosis of this kind can take place; besides, the methods recommended by Calmette, Guérin, Paul, Groth, and others, do not take sufficiently into account the danger of contamination of the vaccine with the virus of foot-and-mouth disease. The risks of infecting children seem indeed to be very small. There is likewise very little prob-

ability of children vaccinated with calf lymph, particularly in the country, transmitting the disease to the domesticated animals; but, in this respect, the possibility is conceivable. On the other hand, it seems that there may be a great danger of the disease spreading from centres in which vaccine lymph is prepared with the calves employed in the process; such calves may well become virus carriers, and, in consequence, distribute eventually enormous quantities of infective material, having in mind the open wounds remaining after collection of the lymph.

It is, therefore, submitted that the Office International des Epizooties should pass a resolution stating that the different States should require, among other control tests upon the vaccine before it is put up for sale, examination by a competent inspector in veterinary matters, with a view to determining the possible existence of the foot-and-mouth virus in it. (*Office International des Epizooties Bulletin*, August, 1930.)

BESREDKA'S METHOD OF ANTI-ANTHRAX VACCINATION.

By R. KARMANN.

BESREDKA vaccinated guinea-pigs and rabbits by rubbing into the shaved skin (cutaneously), first, anthrax Vaccine I, and after six days, Vaccine II. The animals so treated not only survived but also proved to be protected against quantities of anthrax bacilli many times exceeding the lethal dose. The same results were obtained when the vaccines were injected into the skin (intracutaneously). The serum of the guinea-pigs and rabbits vaccinated in either of those ways did not protect other animals against anthrax. Further, Besredka inoculated the vaccinated guinea-pigs and rabbits with virulent anthrax bacilli into the brain, trachea, blood stream, peritoneal cavity, and intestine without making the animals ill, provided that infection of the skin was avoided in introducing the bacilli. Even one thousand times the minimum lethal dose was thus given without serious results. If, however, there was simultaneous infection of the skin the animal died.

Besredka explained the results of these experiments in the following way: Anthrax is an infectious disease of the skin, not of the whole body. The skin alone is susceptible to infection with anthrax, all other organs lacking this susceptibility. In these conditions the anthrax bacilli behave like saprophytes, *i.e.*, they neither cause disease nor confer protection. Only with skin, and indeed only in the layer of reticulo-endothelium, are the bacilli able to multiply and form toxin and thus become harmful later, but only immediately before death can the local disease in the skin develop to a general septicæmia.

As the skin, according to this view, is the only site of anthrax infection, so also it is the sole site of anthrax immunity. Antibodies are not produced, hence guinea-pigs and rabbits, which are specially susceptible to anthrax, resist skin inoculation with Vaccines I and II, and as a result of the vaccination become insusceptible. Animals immunised by cutaneous inoculation show not only a local, but also a general, immunity, since they possess acquired immunity of the skin and also the natural immunity of all the other organs.

Besredka's method of vaccination has been tested in many experiments by different authors, and with varying results.

The present author expresses the opinion that upon the whole the evidence for and against the Besredka method is about equal. His own experiments were intended to answer the following questions:—

1.—Do guinea-pigs survive a double cutaneous or intracutaneous inoculation with anthrax bacilli?

2.—Do guinea-pigs inoculated in this way acquire immunity against anthrax?

3.—Can sheep be rendered immune by such double inoculations?

4.—Do sheep after a single intracutaneous inoculation acquire an adequate immunity against anthrax?

5.—Does the serum of sheep inoculated by the cutaneous and intracutaneous methods contain antibodies detectable by animal experiment?

The results were briefly as follows :—

With regard to question 1 : of 20 guinea-pigs vaccinated cutaneously with Vaccine I only one died from anthrax, and of an equal number vaccinated intracutaneously two died. The surviving guinea-pigs of each of these two tests were given the second vaccine six days later. Of the 19 vaccinated cutaneously four died from anthrax, and of the 18 vaccinated intracutaneously five died.

There survived 15 of the 20 animals vaccinated cutaneously and 13 of the 20 vaccinated intracutaneously. Eight days after the second vaccination these 28 animals were subjected to an immunity test, ten untreated guinea-pigs being used as controls. The result was the death from anthrax of the whole ten controls, and the survival of the 28 vaccinated animals.

The local effect of the vaccine was slight and the swelling disappeared in a few days.

The experiments with sheep were performed as follows : In each of four sheep an area of skin about the size of the palm of the hand on the left side was shaved, cleansed, and dried. Thereafter 8 c.c. of a twenty-four hours' broth culture of Vaccine I was rubbed into the skin with cotton wool. Twenty-four hours later the area of skin treated in this way showed strong congestion, but this disappeared in a few days. The general condition of the animals was not affected.

Six days later 8 c.c. of a twenty-four hour broth culture of Vaccine II was simultaneously rubbed into a shaved area on the opposite side. The local effect was the same as after inoculation with Vaccine I, but two of the sheep died within three days from anthrax. The other two sheep showed for one day diminished appetite but afterwards appeared all right.

At the same time as the above animals, four other sheep were inoculated intracutaneously ; first, each animal was inoculated at several places in the skin with altogether about 0.3 c.c. of a twenty-four hour broth culture of Vaccine I. After twenty-four hours there had developed at each point of inoculation a pea-sized hard nodule, which in a few days disappeared. There was no general disturbance of the health of the animals. After six days they were inoculated intracutaneously at two places in the skin with a twenty-four hour broth culture of Vaccine II, the combined dose for each sheep being 0.15 c.c. These inoculations were also well borne ; the local changes (vesicular and nodular) were the same as after the first inoculation.

As a test of the resulting immunity, ten days after the second inoculation the six inoculated, and at the same time the two untreated control sheep, were inoculated intracutaneously with an anthrax culture which was ten times greater than the lethal dose for a guinea-pig. Both the cutaneously and the intracutaneously vaccinated sheep remained alive, while both the controls died from anthrax, one in two and the other in three days.

The cutaneous and intracutaneous inoculation of sheep with Pasteur's

Vaccine I and Vaccine II therefore conferred a distinctly recognisable immunity against anthrax, but two of the four sheep inoculated cutaneously with Vaccine II died from anthrax.

It would be a great advantage if a sufficient degree of immunity could be conferred by a single intracutaneous inoculation. Unfortunately the author had only one sheep for an experiment to test this possibility. On the same day as the other sheep were inoculated for the second time, as previously described, a fresh sheep was inoculated intracutaneously at two places with altogether 0.15 c.c. of Vaccine II. The local reaction to this inoculation disappeared after six days. The general condition was not disturbed. Ten days later the sheep was tested as to its immunity by intracutaneous inoculation with the same dose of virulent anthrax culture (ten times the minimum lethal dose for a guinea-pig). The sheep remained healthy.

Further experiments were directed to discover whether specific antibodies could be detected in the serum of the sheep that had been inoculated cutaneously and intracutaneously with the Pasteur vaccines. The blood for the antibody tests was drawn off after the intracutaneous immunity test, and immediately after the blood was taken each sheep was inoculated subcutaneously with a dose of virulent anthrax culture, (ten times the lethal dose for a guinea-pig). This inoculation did not cause any apparent disturbance.

The tests were carried out on rabbits, and the results indicated that the cutaneous and intracutaneous inoculation of sheep as a rule does not lead to the formation of recognisable antibodies.

Apparently Besredka's method has not been used in actual practice for the prevention of anthrax in Germany, but in the author's opinion the question of its value is of great scientific practical importance and experiments to test the value of the method ought to be carried out on an extensive scale with the larger domestic animals. (*Zeitsch. für infekt. und paras. Krankh. und Hyg. der Haustiere*, 1931, Vol. 39, pp. 51-69.

EXPERIMENTS BEARING ON THE USEFULNESS OF SURGICAL PROCEEDINGS IN CONNECTION WITH THE INTESTINES OF THE HORSE.

By PROF. P. P. TIMOFCEFF.

It has for long been generally believed that the horse is specially predisposed to traumatic peritonitis. Practical experience and the results of some experiments appear to indicate that the opening of the peritoneal cavity in the equine species is more dangerous than in the other domesticated animals. The selection of the site of operation and the method of closing the laparotomy wound are of importance for the favourable result of surgical interference in the abdominal cavity. In recent years, however, there have been recorded numerous cases which do not conform to the view that surgical opening of the peritoneum of the horse is likely to be followed by a violent peritonitis. Many authors have reported cases in which healing of wounds of the abdominal wall and intestines has taken place promptly. The present author has himself treated 65 cases of penetrating abdominal wounds in horses, and has obtained favourable results in almost all of them. The healing in these cases was not accompanied by any elevation of the temperature. Judging from the literature on the subject, very few attempts to perform

operations on the intestines have been recorded and the results have nearly always been unfavourable. This appears to be not in keeping with the consequences of accidental perforating wounds of the abdominal wall and wounds of the intestine. The author had himself to discover by experiment whether the peritoneum of the horse is in fact specially vulnerable to infection, whether some methods of operation in connection with the abdomen and intestinal canal are practicable, and lastly, whether antiseptic methods are practicable in these operations. He had at his disposal ten experimental horses.

Experiment I.

November 24th, 1929. Subject eight years old, in bad condition. Before the operation temperature normal, no food given, but the diet of the animal had usually been scanty. The field of operation was shaved, washed with warm water containing 5 per cent. lysol, carefully wiped with sterile cloth, then pencilled with tincture of iodine. The animal was cast on the left side and narcotised with chloroform; about 200 c.c. were used, and the narcosis lasted three and a half hours. At the left paralumbar fossa, and about three fingers-breadth from the last rib, an incision 10 cm. long parallel to the ribs was made. The abdominal wall was divided and the head of the cæcum was pulled out. The abdominal wound was plugged with a sterile cloth, saturated with normal saline solution. An incision about 10 cm. long was made in the wall of the intestine and some fæces and a number of parasites were taken out through the wound, and, after rinsing the intestinal wound with warm Rivanol solution (1 in 500), the wound was closed with Lembert sutures and the intestine put back in the abdominal cavity. The abdominal wall was sutured, but the lower fourth of the skin wound was left open. The suture was smeared with Airol paste. The temperature after the operation was always normal and the appetite was not diminished. The wound had healed on the fourteenth day after the operation.

Experiment II.

December 5th, 1929. A gelding eight years old, general condition moderate. An exostosis and a small wound on the right fore fetlock. The preparation for the operation was as in the first case, and there was no preliminary diet. The animal was cast on the right side, and chloroform narcosis lasted two and a half hours (about 25 c.c.). In the region of the flank an incision 15 cm. long was made through the skin and muscle, beginning 2 cm. under the external angle of the ilium and parallel to and 6 cm. behind the last rib. Much hæmorrhage. The peritoneum was ruptured and a loop of the small colon was taken out and isolated with two Doyen forceps. An incision about 12 cm. long was then made through the wall of the bowel and a quantity of fæces was removed through the incision. After the bowel had been washed with Rivanol solution the incision in it was closed with Lembert sutures and put back in the abdominal cavity. The wound in the wall was closed with interrupted sutures. During the next few days there was no swelling at the seat of the operation and the sutures were intact, but afterwards the condition of the animal became worse, the temperature rose, the appetite was suppressed, the respiration was snoring, and the breath had a stinking odour. Death occurred on the seventh day after the operation. The post-mortem examination revealed gangrenous pneumonia (chloroform?), circumscribed peritonitis and necrosis of the muscular tissue at the site of the operation, and suppurative pleurisy on both sides. The wound in the intestine was healed.

Experiment III.

December 13th, 1929. A sixteen years old gelding in bad condition. The operation was performed in Vinsot's apparatus. Laparotomy at the linea alba. The seat of the operation was shaved, wiped with iodobenzine and brushed with tincture of iodine. The chloroform narcosis lasted two hours (about 200 c.c.). The abdomen was opened one to one and a half cm. from the linea alba and a hand-breadth from the ensiform cartilage, the different layers of the wall being cut through in succession, and the incisions 10 or 12 cm. long. For nearly half an hour the different parts of the intestine were carefully felt with the fingers, and during this time some loops of intestine fell out. The peritoneum was not sutured, but the wound in the abdominal wall was closed by interrupted sutures, the distance between the stitches being about 2 to $2\frac{1}{2}$ cm. The sutures were covered with Airol paste and an aseptic dressing was applied to the wound. Healing occurred by first intention and the sutures were removed on the fourteenth day. There was no elevation of temperature and no loss of appetite after first intention and the sutures were removed on the fourteenth day. There was no elevation of temperature and no loss of appetite after the operation.

Experiment IV.

December 15th, 1929. An eight years old gelding. General condition moderate. There was a healing fistula on the left side of the withers. The animal was given 50 c.c. of chloralhydrate, and about the same amount of chloroform by inhalation. Deep narcosis resulted. Preparation as in the previous experiment. The animal was placed in the dorsal position as follows: The fore and hind legs were separated as far as possible and tied to a stick, which was then raised by a strong wire rope attached to the stick at one end and to a pulley in the roof at the other. It is said to be easy to fix a horse in this position when narcotised. The abdominal cavity was opened by an incision about 12-15 cm. long near the linea alba as in the previous case, and about half the length of the cæcum was brought out and incised in the long direction for about 10-12 cm. Some intestinal contents were removed through the wound. The wound in the wall of the intestine was washed with Rivanol and closed with silk Lembert suture. The intestine was again washed with warm Rivanol before being put back into the abdomen. The wound in the wall was closed with interrupted suture. No elevation of temperature or loss of appetite, healing by first intention, and the animal was discharged for work on the seventeenth day after the operation.

Experiment V.

December 17th, 1929. Gelding over 20 years old, in bad condition. Signs of pulmonary emphysema and dilatation of the heart. Preparation as in previous cases. No food before the operation. Chloralhydrate (45 c.c.) by intravenous inoculation and chloroform (about 120 c.c.) by inhalation. Fixation as in the preceding case, and abdomen opened in the same way near the linea alba. The cæcum was pulled out and about 10 cm. of its blind end was excised. Some of the contents were removed. The intestinal wound was closed by tobacco-pouch suture $1\frac{1}{2}$ cm. from the edges. The wound edges were turned in and the suture pulled and knotted. The turned-in edges of the intestinal wound were also united with Lembert suture. The wound in the abdominal wall was closed by interrupted sutures about 1 to $1\frac{1}{2}$ cm. apart. By the fourteenth day the abdominal wound had healed by first intention. Temperature and appetite had remained normal.

Experiment VI.

December 18th, 1929. Gelding about 20 years old. Symptoms of pulmonary emphysema. Animal placed in the dorsal position and combined narcosis as in the previous two cases. Operation near the linea alba, and about 15 cm. was resected from the blind end of the cæcum. Further treatment as in the previous case and result similar—no elevation of temperature or loss of appetite, and healing by first intention in twelve days.

Experiment VII.

December 20th, 1929. Gelding 16 years old. General condition moderate. Preparation as in previous cases and no food before the operation. Combined narcosis by the use of chloralhydrate and chloroform as in previous cases. Opening made in the linea alba, part of the blind end of the cæcum, about 18 cm. long, was resected, and a large quantity of the contents of the bowel removed. Suturing of the bowel wall as in Experiment No. 5, and abdominal wound closed with interrupted sutures. Soon after the operation the horse became restless, and it died after showing symptoms of severe colic. The post-mortem showed that hæmorrhage had occurred, the cæcum being filled with blood. The sutures were intact.

Experiment VIII.

February 15th, 1930. The operation in this instance was on the same animal as the one employed in Experiment I, which had survived enterotomy of the cæcum. Operated upon in the dorsal position. Combined chloralhydrate and chloroform narcosis, and operation near the linea alba. Part of the cæcum, about 15 cm. long, resected. Tobacco-pouch suture of the bowel wound as in Experiment V; the bleeding vessels had to be ligatured. Six hours after the operation the horse got up with difficulty. Temperature 39° C., pulse weak. The animal died on the following day from hæmorrhage, as in Experiment VII.

Experiment IX.

February 27th, 1930. An eight years old mare, in moderate general condition. Spaying had been performed two weeks previously, and the animal was in the way of recovering from peritonitis. Bad appetite. Chloralhydrate narcosis (60 c.c. intravenously). Deep narcosis resulted. Operation performed as in Experiment VIII, and about 10 cm. of the cæcum was resected. The bleeding vessels were most carefully ligatured. Death after twelve days from fibrinous peritonitis.

Experiment X.

March 10th, 1930. A four years old stallion, general condition moderate, no preliminary dieting. Preparation for operation in usual way, and narcosis by intravenous injection of 50 c.c. chloralhydrate. Laparotomy near the linea alba, in the usual position. Resection of about 20 cm. from the point of the cæcum. Suturing as in Experiment V. No dressing applied to the wound. No rise of temperature or loss of appetite. The wound healed by first intention in fourteen days.

SUMMARY.

In the series of experiments the laparotomy was in the flank in two, and near the middle line in eight. In the former much hæmorrhage took place from the abdominal wound, and it was arrested with difficulty. The seizing of the vessels *en masse* with strong artery forceps caused wounding of the abdominal wall. In the author's opinion the local conditions in the region

of the flank are not favourable for uncomplicated quick healing of the wound, as exudation takes place into the deeper tissues and it is difficult to effect drainage. Besides, at this place it is almost impossible to feel the intestines with the fingers. The conditions are more favourable near the linea alba, where no large blood vessels are encountered and the abdominal wall is comparatively thin. In this position one can easily examine the intestines. The experiments show that wounds of the cæcum and colon can heal quickly.

In one case (Experiment V) part of the serous covering of a loop of intestine was probably torn off in resecting part of the cæcum, but in spite of that the animal recovered. (*Archiv. für Tierheilk.*, Vol. 62, 1930, pp. 103-108.)

THE TUBERCULOUS VIRUS.

By A. CALMETTE AND J. VALTIS.

IN 1910 Fontès announced that by using a filtrate of matter from a caseous lymphatic gland of a tuberculous guinea-pig infected with the human type of tubercle bacillus he had succeeded in infecting other guinea-pigs. The liquid used did not contain bacilli or any other stainable element visible under the microscope. Fontès observed further that the infection caused by the filtrate of tuberculous pus did not pass beyond the stage of swelling of the various groups of lymphatic glands, but re-inoculation from these glands to new guinea-pigs produced in the latter more pronounced glandular lesions in which ordinary acid-fast tubercle bacilli were to be found.

Although in the centrifuged filtrate he had not been able to find any other element visible under the microscope, even after staining by the Ziehl method, Fontès supposed that the fact that it caused a typical tuberculosis in animals had to be attributed to Gramophile granules, which had previously been seen and described by Babes, Strauss, and Much. It was supposed that these might be spores, but in reality they were only small protoplasmic masses of a lipid nature.

The discovery made by Fontès was received with sarcasm, and, as it remained unconfirmed and Fontès himself did not push his researches any further, it was soon forgotten.

In 1922, Vaudremer, when studying the varying acid-fastness of tubercle bacilli according to the medium in which they were cultivated, observed that in culture media very poor in nitrogenous material and devoid of glycerine, such as simple potato broth, typical forms of bacilli that were not acid-fast appeared in the depth of the liquid, and among these there were present elements which passed through a Chamberland filter L_3 .

Since 1923 Calmette and Valtis have undertaken further research on this subject. At the outset the experiments made by Fontès were repeated and confirmed. The new experiments soon proved the existence of what has since been called the tuberculous ultravirus—that is, elements invisible with the microscope, but filterable through porous porcelain candles and present in the sputum of consumptives, in the caseo-pus of tubercles, in tuberculous organs, in the circulating blood, in various humours or exudates of patients, and also in the young surface growths of cultures of tubercle bacilli in various artificial media.

The present authors give a detailed account of their method of preparing filtrates from such materials.

Solid material, such as the pulp of organs, is triturated in a mortar with sterile sand, then made into an emulsion by addition of a variable amount of

normal saline (10-50 c.c.), and passed through a previously moistened paper filter. The liquid thus obtained is passed first through a Chamberland L₂ candle and to it there is added a few drops of a twenty-four hours culture of the fowl cholera bacterium, after which it is again passed through a new sterile L₂ candle under pressure of 10 c.m. of mercury, the filtration occupying from 8-10 minutes.

Tuberculous material of other kinds were used in the same way, save for variations in the preliminary steps necessary to prepare the liquid for filtration. In all cases there was a double filtration through Chamberland L₂ candles and some drops of fowl cholera culture were always added to serve as a test of the candles used.

In the case of cultures of tubercle bacilli only young surface growths of six to eight days, grown on Sauton's synthetic medium, were used.

Immediately after filtration about 1 c.c. of liquid is used to seed Martin's broth, Truche's medium, and Veillon's agar (anaerobic). If these media remain sterile after 48 hours the filtrates are used for the inoculations. They remain virulent for eight days in the ice chest and during forty-three days at the temperature of the laboratory if protected from light.

Even after prolonged centrifugation microscopic examination does not reveal, either before or after staining, any visible element.

Filtrates from tuberculous organs, exudates, etc., or from cultures of tubercle bacilli, obtained in the manner already indicated, and which do not contain any elements visible under the microscope, when inoculated into a guinea-pig, either under the skin or into the peritoneum, frequently, but not constantly, determine glandular lesions, generally discrete, but without visible tubercles. In these lesions, which are specially distinct in the tracheo-bronchial glands, one can find on careful searching from the third to the tenth day after inoculation, up to about the eighth week, and rarely after three months, acid-fast bacilli and also granules stainable by Ziehl's method, either isolated or in larger or smaller groups. Around some of these acid-fast bacilli, staining with Borrel's blue reveals very numerous extremely small granules, among which some are coccus-like and others in bacillary form, all devoid of resistance to acids.

After inoculation of filtrates, especially into the peritoneum, many animals die after a time varying from some days to some weeks, without presenting the slightest visible lesion; in the slightly enlarged lymphatic glands in such cases, however, a careful and prolonged examination will often discover a few bacilli. Sometimes the spleen is a little enlarged and the lungs may present small foci of hepatisation. Generally one finds only slight swelling of the glands. When such glands are crushed and injected under the skin of other guinea-pigs the same glandular tumefaction is reproduced, and after six or eight passages in series, sometimes even after three passages, the inoculated animals may show genuine tuberculous lesions with nodules progressing towards caseation. Such lesions, re-inoculable in series, present all the characteristics of chronic tuberculosis. The following experiment is given as an example.

On December 29th, 1925, fourteen bronchial glands of a child dead from tuberculosis, in which tubercle bacilli were abundant, were triturated in a mortar with sterile sand and converted into a fine emulsion by the addition of 50 c.c. of normal saline solution; after 24 hours some culture of the fowl cholera bacterium was added and the material was then passed in succession through two Chamberland L₂ candles, previously tested as to their efficiency. Tubes of broth and of agar seeded with this filtrate remained sterile.

On December 31st, 5 c.c. of the filtrate were injected into the peritoneum of each of two guinea-pigs. One died on the sixth day afterwards without lesions, and it was not possible to find tubercle bacilli in its organs.

The other died on the twelfth day. Its tracheo-bronchial glands were obviously tumefied and contained numerous acid-fast bacilli. None were found in the mesenteric glands.

On January 7th, 1926, 5 c.c. of the same filtrate, which had been kept in the ice chest for eight days, were injected into each of two other guinea-pigs. One died on the second day afterwards, without lesions and without bacilli in its organs.

The other died on the thirteenth day, and bacilli were found in the swollen omental, sub-splenic, and tracheo-bronchial glands.

When one inoculates a normal guinea-pig with acid-fast bacilli evolved from the filterable elements of the tuberculous virus, one observes the same glandular lesions and the same absence of genuine tubercles as after the direct inoculation of filtrates of organs or filtrates of cultures.

By successive passages from guinea-pig to guinea-pig, starting from the filtrate of a culture of bovine tubercle bacilli (Vallée strain), the virulence of the bacilli derived from filterable elements may be increased to such an extent that after five to eight passages they determine caseous lesions identical with those produced by the inoculation of ordinary tubercle bacilli.

For over five years the authors made vain efforts to cultivate the filterable elements of Koch's bacillus. In 1923 Vaudremer claimed to have succeeded, but this was not confirmed in subsequent experiments by others. The present authors, however, claim to have succeeded by using egg medium prepared according to Besredka's method, with the addition to each tube of fresh rabbit serum an extract of ox red corpuscles and three drops of neutral sterile glycerine. It is essential that the pH should be exactly 7.8. The surface of the solid medium of this composition is seeded by depositing on its surface a small quantity of filtrate obtained from the surface growth of a culture from six to fifteen days old, the purity of the filtrate having been previously proved in the manner previously indicated. The seed material must remain as a limpid disc on the surface of the medium, and the inoculated tubes must therefore be moved with care to an incubator at 38° C. During the first eight days the medium appears unchanged and microscopic examinations reveals no visible elements. Starting from the ninth day there is observable a slight turbidity in the upper part of the liquid, which is of a pale orange-yellow colour. This turbidity increases towards the twelfth day, and if at this time one examines a drop of the liquid one discovers numerous long, granular, acid-fast bacilli. Staining by the Gram method of Fontès shows that the granules are disposed laterally along the longitudinal axis of the bacillus. Around these acid-fast bacillary elements in preparations stained with Borrel's blue one finds a considerable number of very fine granules and bacillary elements which do not stain either by Ziehl or by Gram.

Up to the thirtieth day the culture becomes increasingly rich in bacilli, and then these cease to multiply. Sub-cultures from these growths have not been obtained either in the special medium or in the media usually employed.

The bacilli obtained in this way do not appear to be more virulent than the filtrate from which they were obtained. Cultures more than six days old, when inoculated in doses of 10 c.c. subcutaneously to guinea-pigs, do not cause any visible tuberculous lesions, even after eleven months but during the first weeks there is observed the characteristic tumefaction

of the lymphatic glands, and in the pulp of these one frequently finds, especially about the second week, some tubercle bacilli, quite distinct but very rare.

Inoculation of rabbits with the culture of from nine to twenty-three days often causes death in 24-48 hours. Even smaller amounts than 1 c.c. cause striking symptoms of intoxication—paralysis or paresis of the hindquarters or of all four legs, or more or less pronounced opisthotonos. If death does not occur within 48 hours the animal recovers.

It appears that cultures of the ultra-virus produce a toxin that is extremely labile, since it does not appear until the ninth day, when the very fine granular non-acid-fast elements predominate, and apparently it disappears after the twenty-third day, when the granular elements seem to undergo intense lysis and are replaced by the acid-fast bacillary elements.

It is suggested that the effects of this toxin on the rabbit, and the sudden deaths which have frequently been observed in new-born children of tuberculous women, may be of the same nature. In such cases in children it is impossible to discover post-mortem any lesion to account for the death.

CONCLUSIONS.

The authors believe that in consequence of recent discoveries the classical conceptions regarding the pathogenesis of tuberculous infection must be modified.

In future one must admit that tuberculosis, characterised by the presence in various organs (principally in the lymphatic glands and lungs) of giant cells and tuberculous nodules containing Koch's bacilli, more or less virulent and re-inoculable in series in susceptible animals, represents only the chronic form, or the terminal phase of an infection realised at first by an ultra-virus, the elements of which present a very special type of evolution.

These elements, at first invisible under the highest powers of the microscope, and capable of passing through filters, make their appearance in the lymphatic glands and multiply there. In appropriate artificial media they also multiply in the form of a cloud of extremely small particles, and later as caseous-like granules, some of which give origin to very fine bacilli, which after growth in length divide transversely, and by acquiring resistance to acids become the veritable bacilli.

Until their last bacillary stage, as long as they are not yet adapted to the parasitic life in a susceptible organism, the elements originating from the ultra-virus are quite unable to determine the formation of tuberculous nodules, that is to say they are not tuberculigenic. On the other hand, during a brief period in their evolution and in certain natural or artificial media, they are capable of producing a very active toxin, which appears to have a great affinity for the cells of the central nervous system. They can thus cause rapidly fatal disorders, especially in young animals and infants. When they do not thus cause death the evolution proceeds without any serious symptom on the part of the host until there is produced a bacillary parasitism, which may be quite well tolerated, or which may provoke various pathological manifestations of which the tuberculous origin has not yet been demonstrated, though suspected by clinicians.

One would reserve the word tuberculosis or, better, bacillosis, for the generalised or localised infections which are characterised by the formation of tuberculous nodules or tubercles, and which ordinarily run a chronic course. (*Annales de l'Institut Pasteur*, T. 44, 1930, pp. 629-658.)

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